The Human Cortical Dental Pain Matrix

Neural Activation Patterns of Tooth Pain investigated with fMRI

Thesis

presented to the Faculty of Arts

of

the University of Zurich

for the degree of Doctor of Philosophy

by

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Accepted in the spring semester 2009

on the recommendation of

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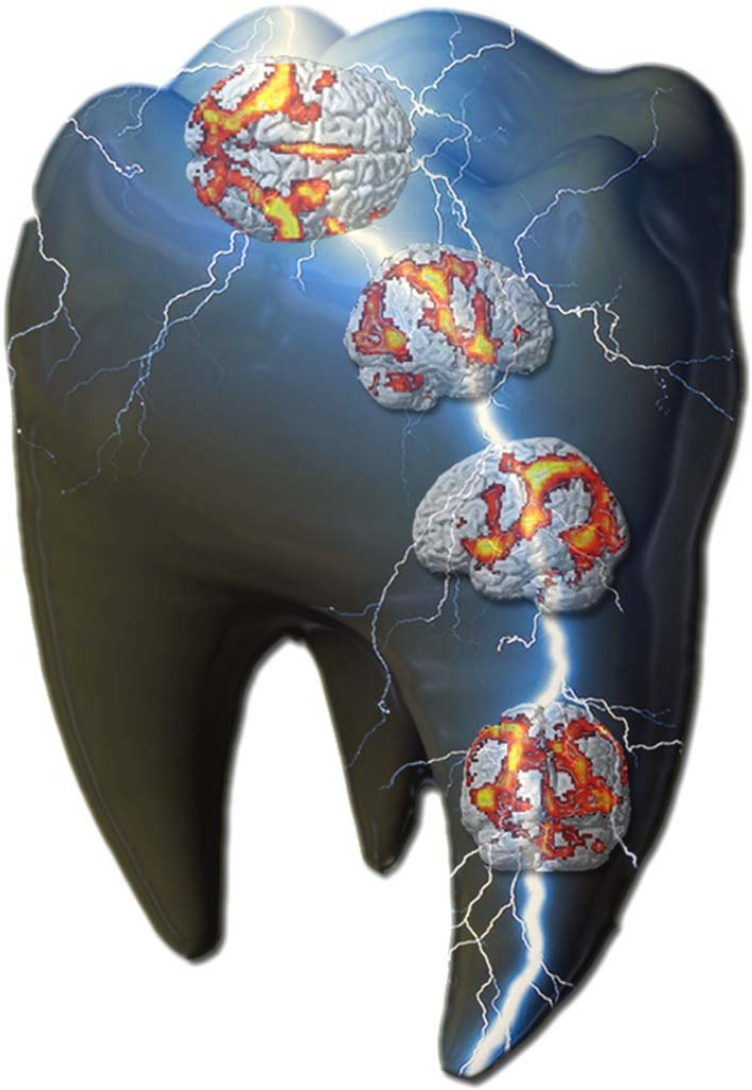
Zurich, 2011



…the authors "art-like" interpretation of a human brain under tooth pain…

CONTENTS

SUMMARY

The knowledge and concomitant understanding regarding the neural correlates of a human pain perception has reached an astonishing level since the advent of neuroimaging techniques. However, despite the fact that tooth pain is very common among the population, the known fundamentals according to the respective brain mechanisms are next to nought compared with peripherally related cortical pain processing.

Along the way to investigate dentinal hypersensitivity, a specific clinical tooth pain condition, this PhD thesis, therefore, aimed at elucidating brain response mechanisms underlying experimentally induced painless and painful dental sensations in healthy human subjects. This approach has been chosen because almost nothing was known in terms of brain responses related to tooth sensations, thus, it was pivotal to first consider this aspect by investigating healthy people before an accurate interpretation of patient's responses was possible.

Experiment 1 was conducted to investigate basic brain mechanisms due to tooth sensations ranging from non-nociceptive to nociceptive with a specific focus on inter individual differences. A second aspect considering this initial experiment was linked to the question, whether our experimental setup is accurately working over a complete study timeframe.

In experiment 2, the question was addressed whether the trigeminal system follows a tendency for lateralization as it is known from the peripheral somatosensory system, with a classification in a lateral and medial nociceptive circuitry scheme.

The probably most important aspect of a pain experience refers to the question: How much does it hurt? Thus, experiment 3 was conducted in order to elucidate relevant brain regions specifically coding for the differentiation of tooth sensations, ranging from painless to painful.

To summarize, the present results indicate that neural correlates of trigeminal mediated somatosensory inputs are largely corresponding with what is known from the peripheral somatosensory system. However, the data also point to some peculiarities, which apparently indicate a different and specified functionality within the human trigeminal somatosensory system. The outcomes of the presented experiments therefore serve as important fundament to a better understanding with respect to the neural underpinnings of general tooth pain as well as pain arisen from dentinal hypersensitivity.

ZUSAMMENFASSUNG

Das Wissen - und damit einhergehend - das Verständnis der neuronalen Grundlagen von Schmerz, hat ein erstaunliches Niveau erreicht, was nicht zuletzt auf den Einsatz moderner Bildgebungsverfahren zurückzuführen ist. Interessanterweise ist die Untersuchung von Zahnschmerz - bezogen auf die zugrundeliegenden neuronalen Mechanismen - marginal, obschon es sich dabei um eine sehr häufige und verbreitete Schmerzform handelt.

Im Verlauf eines interdisziplinären Projektes zur Untersuchung der dentinalen Hypersensitivität, eines klinisch relevanten Schmerzproblems, war das Ziel dieser Dissertation herauszufinden, wie Empfindungen ausgehend vom Zahn - wozu auch Zahnschmerz gehört - kortikal repräsentiert sind. Dabei ging es primär um die Untersuchung kortikaler Prozesse von experimentell induzierten, schmerzlosen und schmerzhaften Zahnreizungen bei gesunden Kontrollpersonen. Dieses Vorgehen wurde gewählt, weil nur wenig über Zahnschmerz spezifische Hirnmechanismen bekannt war und wir es als fundamental erachteten, zuerst bei gesunden Personen relevante Grundlagen aufzeigen zu können, damit eine akkurate Beurteilung von Patientendaten möglich wird.

Experiment 1 war demzufolge ausgelegt, um basale Hirnaktivierungen bei gesunden Versuchspersonen im Hinblick auf schmerzlose und schmerzhafte Zahnreizungen, aufzeigen zu können. Ein weiterer wichtiger Aspekt dieses Experimentes bezog sich auf die Frage, ob sich unser Stimulationssetup über eine ganze Studie bewähren kann.

Experiment 2 war ausgelegt um die Frage zu klären, ob das trigeminale somatosensorische System einer Unterteilung in ein laterales und mediales Untersystem folgt, so wie dies von Untersuchungen des peripheren somatosensorischen Systems bekannt ist.

Die wichtigste Frage im Zusammenhang mit Schmerz ist wohl: wie stark sind die Schmerzen? Daher wurde Experiment 3 mit der Fragestellung durchgeführt, welche Hirnregionen spezifisch für die Kodierung unterschiedlicher, schmerzloser und schmerzhafter Reizintensitäten am Zahn verantwortlich sind.

Summiert implizieren die gefundenen Ergebnisse, dass das trigeminale und das periphere somatosensorische System hinsichtlich ihrer neuronalen Aktivierungsmuster, weitestgehend korrespondieren. Jedoch fanden sich auch Hinweise, die eine mögliche Sonderstellung des trigeminalen somatosensorischen Systems nahelegen. Die gewonnen Daten bilden daher ein

wichtiges Fundament betreffend neuronaler Aktivierungsmuster von Zahnschmerz generell, sowie dem Schmerz, ausgelöst durch dentinale Hypersensitivität.

PREFACE

With the beginning of my PhD thesis I would like to give a short synopsis of its initial position as well as some crucial aspects in terms of experimentally evoking tooth pain in healthy human subjects.

Originally, the aim was to elucidate neural correlates of dentinal hypersensitivity (DH), a significant clinical problem that causes considerable concern for both, patients and dental professionals. It is described as short, sharp and pricking pain arising typically - but not necessarily - from exposed dentine in response to thermal, chemical, tactile or osmotic stimuli whereas an incidence rate between 4 to 74% is reported (Bartold, 2006; Orchardson and Gillam, 2006; Sykes, 2007).

Because of the manifold challenges, this project was (and still is) multidisciplinary disposed including the *Center for Dental Medicine* and the *Department of Neuropsychology,* both from the University of Zürich*,* the *Automatic Control Laboratory* and the *Institute of Biomedical Engineering*, both from the Swiss Federal Institute of Technology, Zürich. From the industry side, we were in the lucky position to have *GlaxoSmithKline*, *Consumer Healthcare Division* on board. The project initially aimed at developing 1) a rationale that allows to investigate neural and psychological fundamentals of DH and 2) to construct a stimulation apparatus that enables to provoke controlled and standardized sensations of DH in an fMRI environment. The first and most important step however, was to clarify the question about brain specific processes due to tooth pain in general, as no published literature existed. Hence, we firstly focused on designing a stimulation setup to study healthy subjects by inducing tooth pain experimentally, while measuring respective brain activity in a MR scanner. Because of the technically challenging approach, it took us about 10 months to dissipate a working system not interfering with the MR environment and at the same time working accurate and reliable across subjects.

During this thesis, we will see that pain still remains enigmatic, although decades of intensive research in both experimental as well as clinical domains have been conducted (Craig, 2003). Further on, inducing pain in healthy volunteers leads inevitably to critical questions with respect to ethical values and moral obligations. Certainly, human experiments are conducted under clearly defined restrictions and rules but nevertheless it is

pain that is explicitly evoked in human subjects and even worse in our case; we intended to administer the pain in a - let's say - rather private and intimate area: the teeth.

When I started this PhD thesis, I was far from being aware about the complexity of all these inherently problematic aspects and learnt quickly; administering tooth pain in a controlled way to a human subject is difficult and demanding. Also testing and validating the stimulation setup generally and specifically in an MR environment was very challenging from a technical perspective. Fortunately, the stimulus delivery device itself had been classified as clinical approved and was expected to work, but it has never been used to provoke painful tooth sensations. Finally, the crucial remaining question was: are there enough "maniacs" who would trust us sufficiently and allow applying the pain-paradigms we had developed. Since, without these brave volunteers, we would not have been able to disentangle the question how toothache is represented within the human brain and this point was absolutely scaffolding for the running project.

1. INTRODUCTION

Can we conquer pain?

This - at first glance - astonishing title of a review paper by Scholz and Woolf, (2002) can still be taken seriously as even after decades of investigating pain in its different facets and an abundance of published literature, lots of the inherent mechanisms remain unclear or even unknown. An extraordinary dichotomisation reflects the dilemma within the pain research field. On the one hand, exciting progress has been made by dissecting out the complex molecular and cellular mechanisms that operate along the sensory pathways and finally generate those neural signals we finally interpret as pain (Basbaum et al., 2009). On the other hand, there is the human being, suffering from pain as it produces severe distress and mostly disrupt the quality of live in significant manner. Aggravatingly to consider is that much of currently available treatment is only partially effective and often causes many side effects (Sindrup and Jensen, 1999; Basbaum and Julius, 2006). And there is further this partly unresolved issue of the subjectivity of a pain experience as - especially when the pain turns to be chronic - no one reacts in the same manner what additionally makes it so difficult in terms of an accurate treatment approach (Scholz and Woolf, 2002). Summarizing these mentioned aspects, there is still a need for wider knowledge dissemination about the underlying pain mechanism and how to deal as effective as possible with these issues (Sessle, 2000; Scholz and Woolf, 2002).

But pain is at the same time very important and plays a crucial role in human existence, as it serves as a warn signal of potentially tissue damage that can harm the organism's integrity. There are many different pain characteristics but broken down to the fundamentals, the underlying peripheral mechanisms are very similar (Scholz and Woolf, 2002; Tracey and Mantyh, 2007). Thus, the individual "colorization" of the pain quality is created within neural circuitries of really complex structure that mainly account also for the subjectivity of pain (Peyron et al., 2000). Astonishingly, pain is often employed as single word or general descriptor of an essentially "multi-factorial entity". It clearly contains both, pure sensory as well as psychological facets and finally ends as a holistic experience that can be sub-classified into four main dimensions, namely a sensory-discriminatory, an affective-emotional, a cognitive and a behavioural component (Sessle, 2000; Tracey and Mantyh, 2007). Important to note is that these different components interact and modulate each other so closely that

none of them can be considered separately. They have to be considered and interpreted as the aforementioned holistic and multi-factorial entity. Because of this interacting diversity, it is so difficult to "fight the beast" as finally every pain status is closely related to the very subjective characteristic of a human being, that is, biologically spoken: a complex and multidimensional acting organism and therefore, all the efforts in the different and very specific realms of the pain research field aim at understanding both, the specific biological pain mechanisms as well as the complex neuro-psychological interaction what finally reflects the accessible epiphany of pain (Peyron et al., 2000; Scholz and Woolf, 2002; Broggi, 2008).

The face and mouth represent sites of some the most common pains in the body (Sessle, 2000) and almost everyone agrees that it is of highly tantalizing and unpleasant valence. Especially dentinal hypersensitivity, a common but somewhat enigmatic condition in clinical dental practice, seems to unify physical and psychological aspects in characteristic manner (Dababneh et al., 1999; Orchardson and Gillam, 2006). The condition is described as short and sharp pain caused by eating, drinking, brushing and sometimes even breathing (Dababneh et al., 1999) and is therefore accompanied by a high probability of unpleasant live affecting side effects. Towards therapeutic interventions, Dababneh et al. (1999) pointed out, that beside haemorrhoids, few other diseases are treated by so many and extremely varied agents and formulations. Professionals are confused about specific diagnosis, aetiology and mechanisms of dentinal hypersensitivity and some practitioners doubt to be capable of managing the condition effectively (Orchardson and Gillam, 2006).

This thesis focused not directly on dentinal hypersensitivity but aimed at developing a rationale that allowed from a neuronal perspective the continuing examination of the disease by trying to elucidate characteristic brain response patterns due to tooth sensations ranging from non painful to painful by means of electric stimulation of different teeth. Functional magnetic resonance imaging (fMRI) has been chosen as the most accurate method to study the brain functions under tooth pain. The present findings, firstly, give an insight of cerebral mechanisms associated with the different facets of human tooth pain and secondly, provide the fundamentals for a deeper understanding of dentinal hypersensitivity in order to a possible unravelling of this painful clinical condition. Further on, the present work provides a better understanding of trigeminal related somatosensory input to the human brain that may also allow understanding other clinical conditions derived from the orofacial region what finally facilitate a more successful treatment of patients.

2. THE HUMAN PAIN SYSTEM

2.1 OVERVIEW

Pain normally serves as an alarm system, activated in response to impending damage to the organism. But as Craig, (2003) provocatively pointed out: Pain remains an enigma. This strong statement can be ascribed to the systems inherent complexity beginning with a potentially damaging peripheral (painful) event over several transmitting cascades and finally, ending in a conscious awareness of the subjectively colored pain.

Following, a shallow framework will be given in order to provide a better understanding form where pain usually starts, namely the periphery. Basic principles and illustrations of nociceptive, inflammatory and neuropathic pain open the journey into the complicated world of human pain followed by a summary of the paths from the periphery to the brain.

2.1 BASIC PAIN MECHANISMS FROM THE PERIPHERY TO THE BRAIN

Basically, pain emerges in the periphery by hurting the cruciate ligaments during football or by tipping on a hot plate, or even worse, as a side effect of a more severe disease like lung cancer or AIDS. However, the induced mechanisms differ in some aspects, depending on the pain origin and the level of threatening the pain is interpreted by the suffering subject.

Theoretically, the peripheral system can be divided into three more or less distinct sub-systems, namely nociceptive, inflammatory and neuropathic pain (Fig. 1). Physiologically, small diameter fibers (primary afferent Aδ and C) transmit the physiological status from various tissues of the whole body, including mechanoreceptors, nociceptors, thermoreceptors, and osmoreceptors. They terminate monosynaptically on Lamina I projection neurons of the spinal dorsal horn, from where connections to the cortex over several relay stations ensure a highly specified, integrated and finally subjective perception of pain. The basic principles of these circuitries are schematically described in figure 2.

Figure 1

Schematic of nociceptive, inflammatory and neuropathic pain principles.

a)

Noxious stimuli are transduced into electrical activity at the peripheral terminals of unmyelinated C-fibers and/or thinly myelinated A-δ fibers by specific receptors sensitive to heat, acid, mechanical and cold stimuli. From here, the activity is conducted over the spinal cord and central pathways to the cortex. Here, the pain sensation as an integer experience is created.

b)

Chemical mediators, released by damaged tissue, inflammatory and tumor cells are creating an inflammation that activates/modifies stimulus response properties of nociceptor afferents.

c)

Conditions which affect the peripheral nervous system, as in carpal tunnel syndrome, traumatic injuries of the spinal cord or the cortex after stroke can cause neuropathic pain. This is characterized by a combination of pain and neurological deficits (Figure from Scholz and Woolf, 2002 modified by the author).

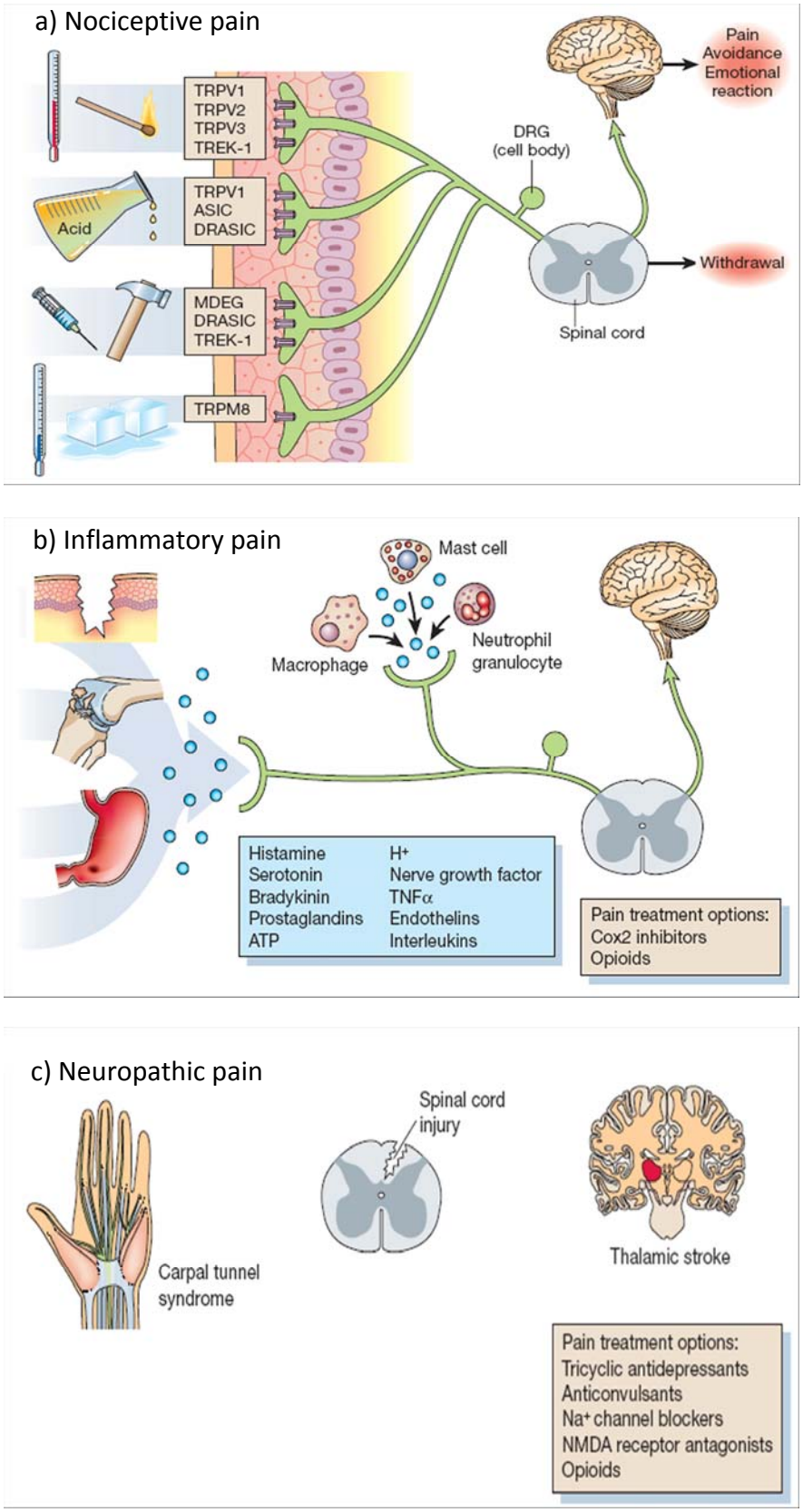
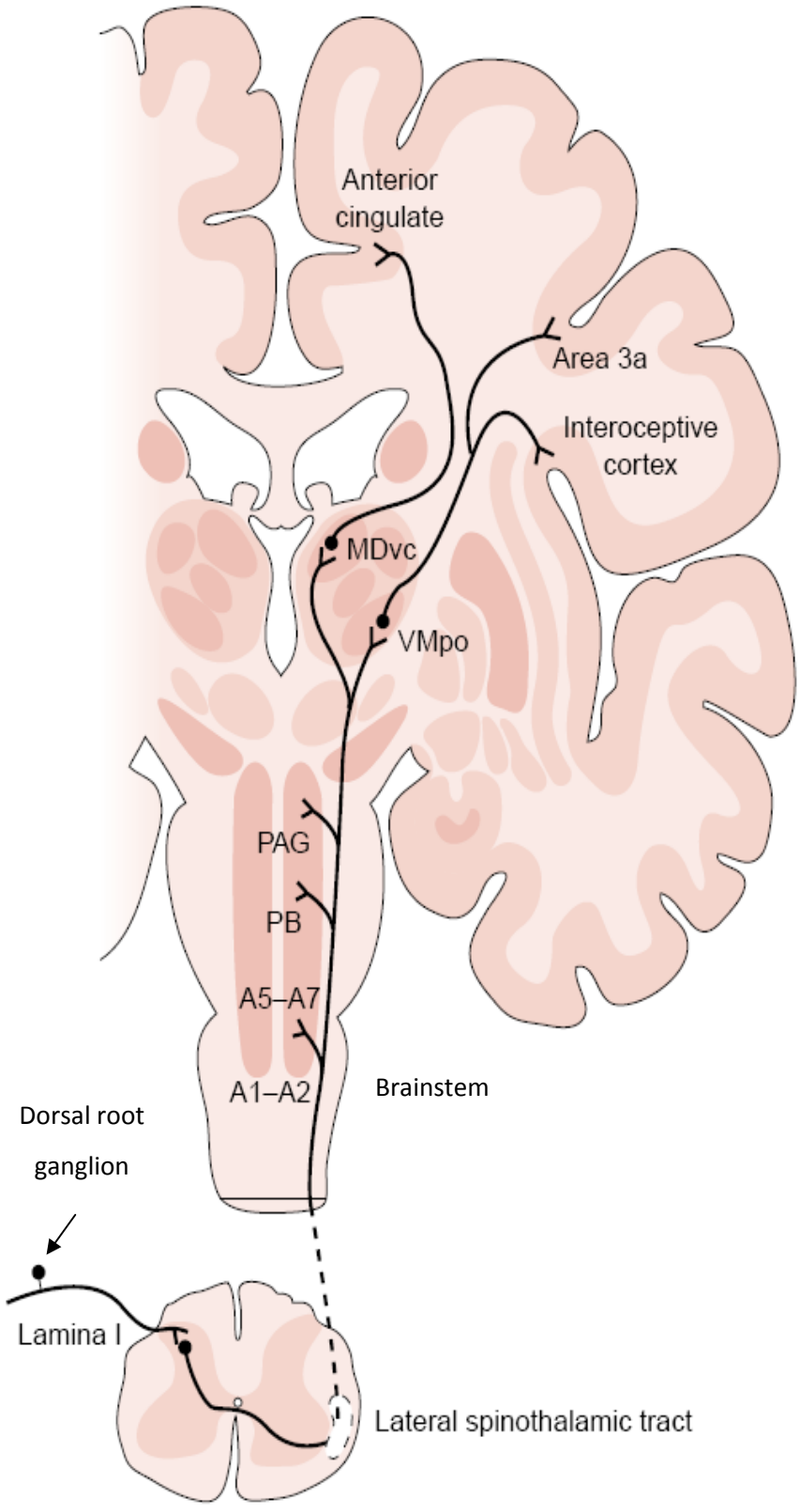


Figure 2

Functional schematic of the projections from the periphery to the human cortex.



Modality-specific lamina I neurons project first to autonomic sites in the spinal cord (not shown) and to the brainstem [including the noradrenergic cell groups A1–A2 and A5–A7, the parabrachial nucleus (PB) and the periaqueductal gray (PAG)]. In primates, lamina I neurons also project by way of the crossed lateral spinothalamic tract to two sites in the thalamus: the posterior part of the ventral medial nucleus (VMpo) and the ventral caudal part of the medial dorsal nucleus (MDvc). The VMpo provides modality-specific sensory representation of the physiological condition of the body in interoceptive cortex at the dorsal margin of the insula, and it sends a corollary projection to area 3a in the sensorimotor cortex. The MDvc integrates lamina I input with brainstem homeostatic activity (from PB and PAG) and produces a specific behavioral drive in limbic motor cortex as well as subdivisions of the cingulate cortex (Figure from Craig, 2003 modified by the author).

2.2 THE SENSATION OF PAIN

The perception of pain due to a severe clinical pain state or an acute injury undergoes substantial processing from the respective peripheral origin to cortical levels and finally, ends in the conscious experience of pain that further submit an accurate integration and reaction (Figures 3a and 3b). Localisation, intensity, unpleasantness, feeling of suffer or grief, the constraint to escape the pain sensation...all this facets are managed immediately and several highly specified response and inhibitory mechanisms have to be induced to prevent the organism from more damage and to initiate respective steps in order to a optimal convalescence. (Craig, 2003).

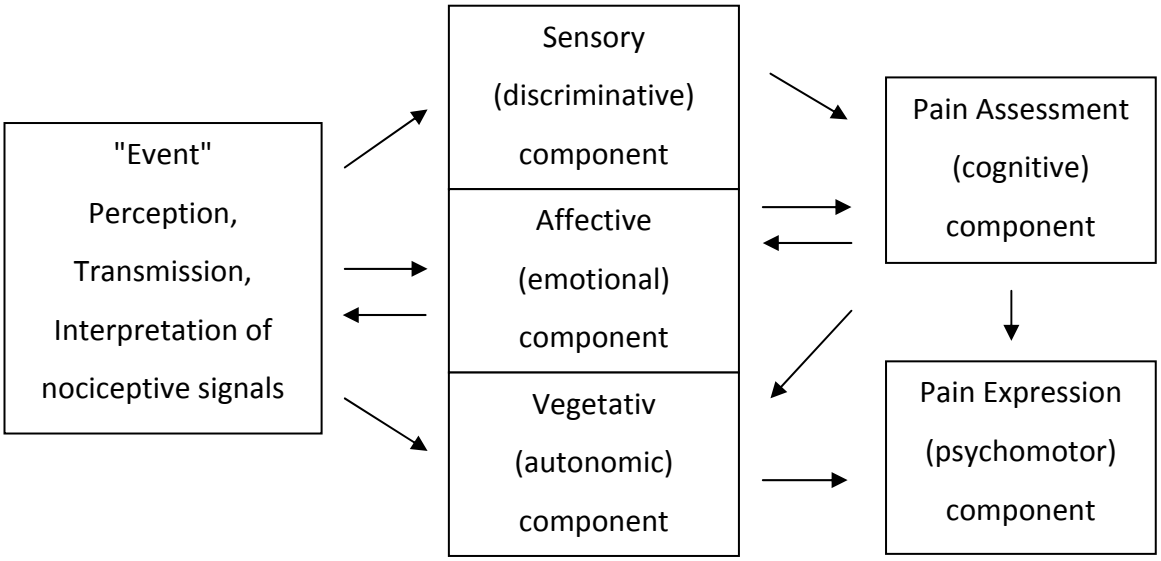


Figure 3a illustrates the relevant components following a painful event or a chronic pain state. Sensory, affective and vegetative subsystems are all affected and closely related to each other. The level of how much each subsystem contributes to the holistic experience depends on subjective and objective elements. A ruptured Achilles tendon can tremendously hurt but after surgery, the outcome is mostly really good and the person knows that fact. On the other hand, pain derived from a cancer disease may not hurt that much, but due to affective influences, such a pain can cause much more harm than the principally worse pain of the ruptured Achill tendon. Both pain states are accompanied by very specific and situation depended factors that influence the perception and related interpretation of a pain experience (see also figure 3b).

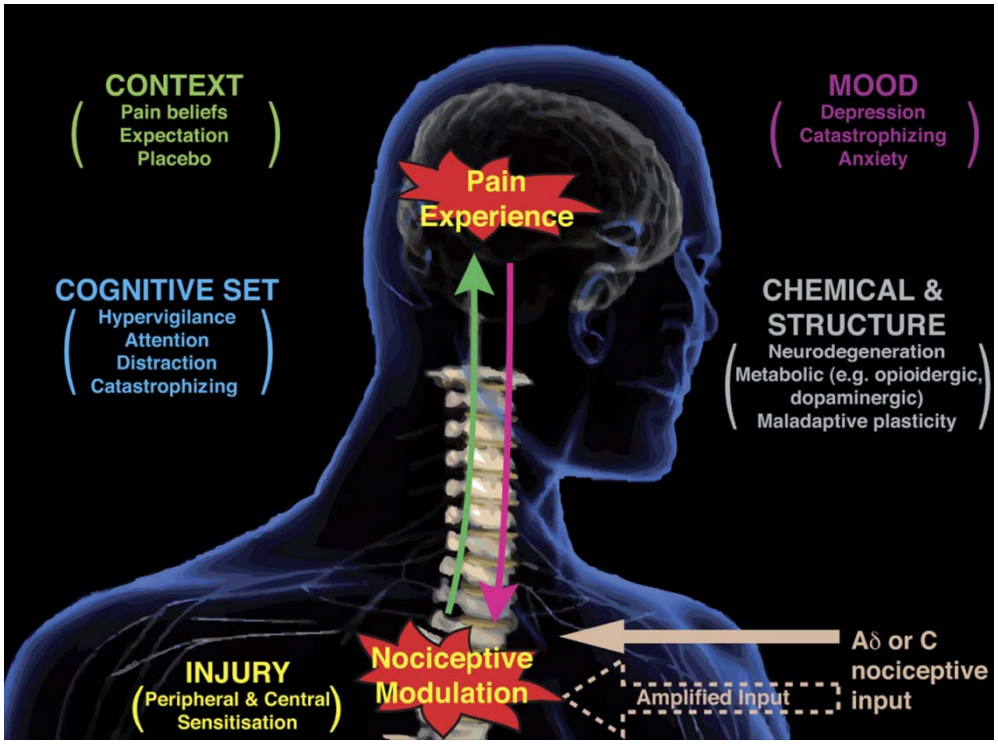


Figure 3b illustrates different relevant components of factors known to influence nociceptive inputs to amplify, attenuate and "color" the pain experience (Figure from Tracey and Mantyh, 2007).

Figure 3a and 3b simplify highly complicated and interacting processes but summarize the fundamentals of an organism being exposed to a painful event or a chronic pain state that has to be integrated to a holistic pain experience and a succeeding appropriate reaction. Another important factor (difficult to cover with illustrations and graphics) is related with the subjectivity of pain as no human reacts identical when experiencing (experimentally evoked) pain (Craig, 2003) nor in clinically related pain states (Keogh and Herdenfeldt, 2002). The challenge of experimental pain research is therefore, to create paradigms and pain-models of highest possible homogeneity in terms of context, the cognitive set and mood (Fig 3b) and to reduce "side effects" like fear and agitation as much as possible. Of course, if one is interested in those facets, then the paradigms have to be adapted in an appropriate way. Independent on which factor is experimentally targeted, it is of immense importance to familiarize volunteers with the stimulation environment, what in fact means a circumstantial psychophysical assessment prior to the effective investigation, irrespective of the deployed methodology. This encompasses the careful explanation of the setup and the applied technologies which is really time-consuming, but in the end, it pays off. Volunteers who

exactly know what will happen and what is expected from them are much more settled and therefore, better results are the reward of the experimenter. The advantage of a carefully conducted psychophysical assessment is further, that it allows getting an impression of how to treat a person ideally, as well as being able to exclude possibly inconvenient volunteers.

3. THE TRIGEMINAL SYSTEM

To understand how the brain processes tooth pain or generally spoken, sensations from the dental and periodontal apparatus, an introduction of the trigeminal system will provide some relevant information.

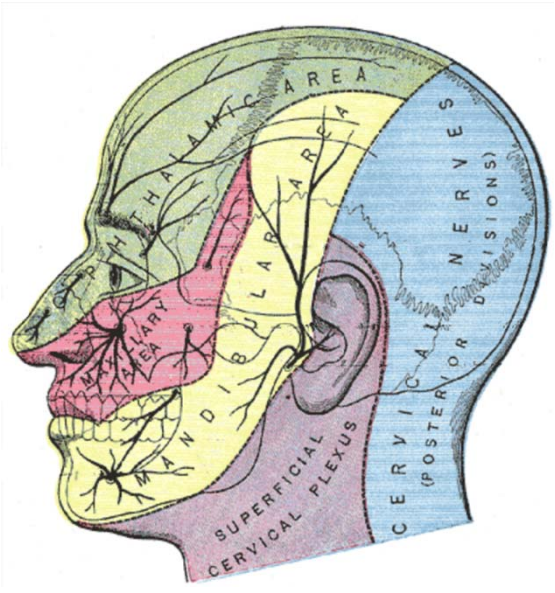
3.1 ANATOMICAL BASICS

From all cranial nerves, the trigeminal is the largest and basically the main sensory nerve of head and face. It emerges from the side of the pons containing a small motor and a large sensory root (Fig 4).

A complicated network subdivided into basically three sensory branches (ophthalmic, maxillar and mandibular), one smaller motor branch and four associated nuclei (motor, principal or main, mesencephalic, and spinal) constitute the trigeminal nerve system (Figure 4). Hence, it can be described as a mixed nerve including motor and sensory functions which afford masticatory and craniofacial reflex function and processes touch, nociception, temperature and proprioception from the face, facial and masticatory muscles, the temporomandibular joint and the intraoral cavity including teeth, tongue and the oral mucosa(Usunoff et al., 1997; Marani and Usunoff, 2000; Sessle, 2006; Broggi, 2008).

Figure 4

Innervation areas of the face covered by the three trigeminal branches. Ophtalmic = green, maxillar = red, mandibular = yellow. The superficial cervical plexus and the cervical nerve do not belong to the trigeminal but are given in terms of completeness (Figure from Henry Gray. 1918. Anatomy of the Human Body: Bibliographic Record and modified by the author).



3.2 SUBDIVISIONS OF THE TRIGEMINAL NERVE

The **ophthalmic nerve** (Fig 4 and Fig 5), also sometimes termed the first division of the trigeminal, is a pure sensory nerve. Of the three divisions of the trigeminal, it is the smallest nerve and arises from the upper part of the semilunar ganglion as a short, flattened band, about 2.5 cm long, which passes forward along the lateral wall of the cavernous sinus, below the oculomotor and trochlear nerves; just before entering the orbit, through the superior orbital fissure, it divides into three branches, lacrimal, frontal, and nasociliary. The ophthalmic nerve is joined by filaments from the cavernous plexus of the sympathetic, and communicates with the oculomotor, trochlear and abducent nerves. It supplies branches to the cornea, ciliary body, and iris, further to the lacrimal gland and conjunctiva, to parts of the mucous membrane of the nasal cavity and to the skin of the eyelids, eyebrow, forehead, and the nose (Henry Gray. 1918. Anatomy of the Human Body: Bibliographic Record; Netter and Dalley, 2000; Nieuwenhuys et al., 2007).

The **maxillary nerve** (Fig 4 and Fig 5), or second division of the trigeminal, is also a pure sensory nerve and is intermediate, both in position and size, between the ophthalmic and mandibular divisions. It begins at the middle of the semilunar ganglion as a flattened plexiform band, passing horizontally forward, leaves the skull through the foramen rotundum, where it becomes more cylindrical in form, and firmer in texture. It then crosses the pterygopalatine fossa, inclines lateral on the back of the maxilla, and enters through the inferior orbital fissure; it traverses the infraorbital groove and canal in the floor of the orbit, then appears upon the face at the infraorbital foramen. At its termination, the nerve lies beneath the quadratus labii superioris, and divides into a leash of branches which spread out upon the side of the nose, the lower eyelid, and the upper lip, joining with filaments of the facial nerve (Henry Gray. 1918. Anatomy of the Human Body: Bibliographic Record; Netter and Dalley, 2000; Nieuwenhuys et al., 2007). The posterior superior alveolar branches (or posterior superior dental branches) arise from the trunk of the nerve just before it enters the infraorbital groove (Figure 4). Generally there are two in number, but sometimes arise by a single trunk (Nieuwenhuys et al., 2007). They then descend on the tuberosity of the maxilla and give several twigs to the gums and neighboring parts of the mucous membrane of the cheek. Then they enter the posterior alveolar canals on the infratemporal surface of the maxilla and passing towards the substance of the bone, communicate with the middle superior alveolar nerve, and give off branches to the lining membrane of the maxillary sinus

and three twigs to each molar tooth. These twigs enter the foramen at the apices of the roots of the teeth.

The middle superior alveolar branch is given off from the nerve in the posterior part of the infraorbital canal, runs downward and forward in a canal in the lateral wall of the maxillary sinus to supply the two premolar teeth. It forms a superior dental plexus with the anterior and posterior superior alveolar branches.

The anterior superior alveolar branch is given off from the nerve just before its exit from the infraorbital foramen (Fig 4) and descends in a canal in the anterior wall of the maxillary sinus fro where it divides into branches which supply the incisor and canine teeth. It communicates with the middle superior alveolar branch, and gives off a nasal branch, which passes through a canal in the lateral wall of the inferior meatus, and supplies the mucous membrane of the anterior part of the inferior meatus as well as the basic of the nasal cavity.

The **mandibular trigeminal nerve** (Fig 4 and Fig 5) is the largest of three divisions and contains two roots: a large, sensory root proceeding from the inferior angle of the semilunar ganglion, and a small motor root (the motor part of the trigeminal), which passes beneath the ganglion, and unites with the sensory root, just after its exit through the foramen ovale. Immediately beneath the base of the skull, the nerve gives off from its medial side a recurrent branch (nervus spinosus) and the nerve to the Pterygoideus internus, and then divides into two trunks, an anterior and a posterior. The mandibular nerve supplies teeth and gums of the mandible, the skin of the temporal region, the auricula, the lower lip, the lower part of the face, and the muscles of mastication. It also supplies the mucous membrane of the anterior two-thirds of the tongue.

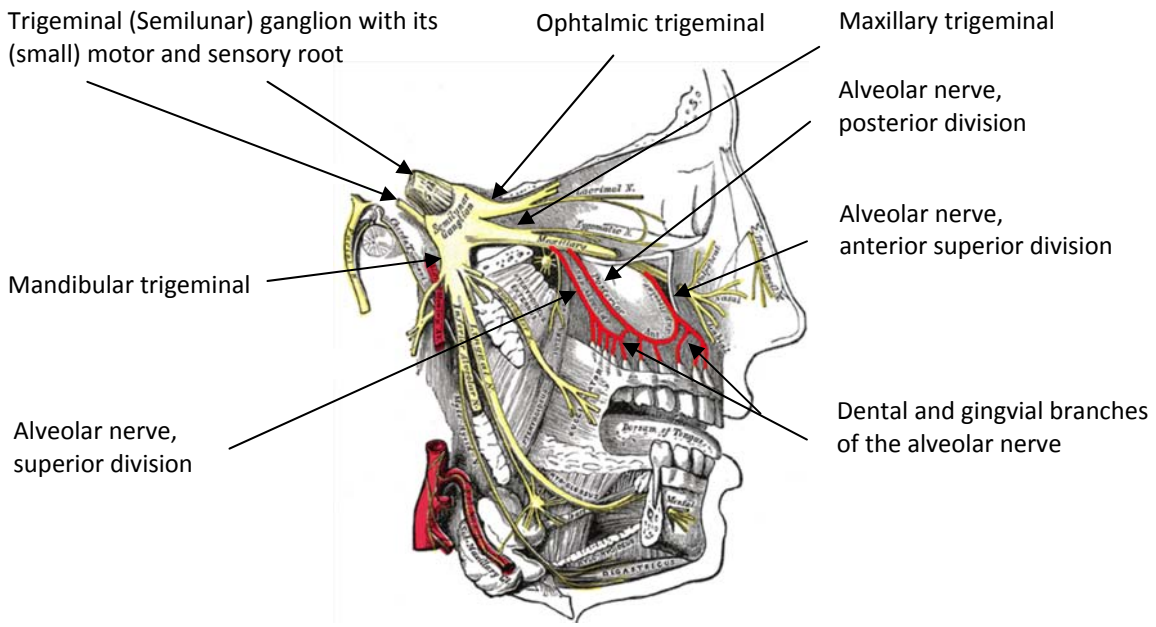


Figure 5 illustrates the trigeminal nerve with its main sensory and the small motor root. Ophtalmic, maxillar and mandibular represent the three main sensory branches. From the maxillary part, the alveolar nerve divert into a superior, posterior and anterior superior division which innervate over dental and gingvial branches the maxillar teeth and gingvia, respectively (Figure from Henry Gray. 1918. Anatomy of the Human Body: Bibliographic Record, modified by the author).

3.3 THE ROLE OF THE BRAINSTEM WITHIN THE TRIGEMINAL SYSTEM

In contrast to the spinal somatosensory system the primary afferent cell-bodies of the trigeminal system are not located in the dorsal root ganglia (Fig 2) but directly within the central nervous system (Fig 6).

Somatosensory fibers of the trigeminal nerve enter the pons through the sensory root from which they distribute to both the principal sensory and spinal trigeminal nuclei. Many of these fibers (A-β, A-γ, A-δ and C-fibers) project through the pons and distribute into the principal sensory and spinal trigeminal nuclei and their subdivisions (Usunoff et al., 1997; Sessle, 2006; Nieuwenhuys et al., 2007).

Thin components of these fibres (A-δ and C-fibers) descend into the spinal tract, which continues as Lissauers dorsolateral fasciculus on top of the dorsal horn. They then terminate in parts of caudal and spinal trigeminal nuclei and in the upper cervical dorsal horn. Fibres of the mandibular trigeminal division descend into the dorsal part of the tract whereas the

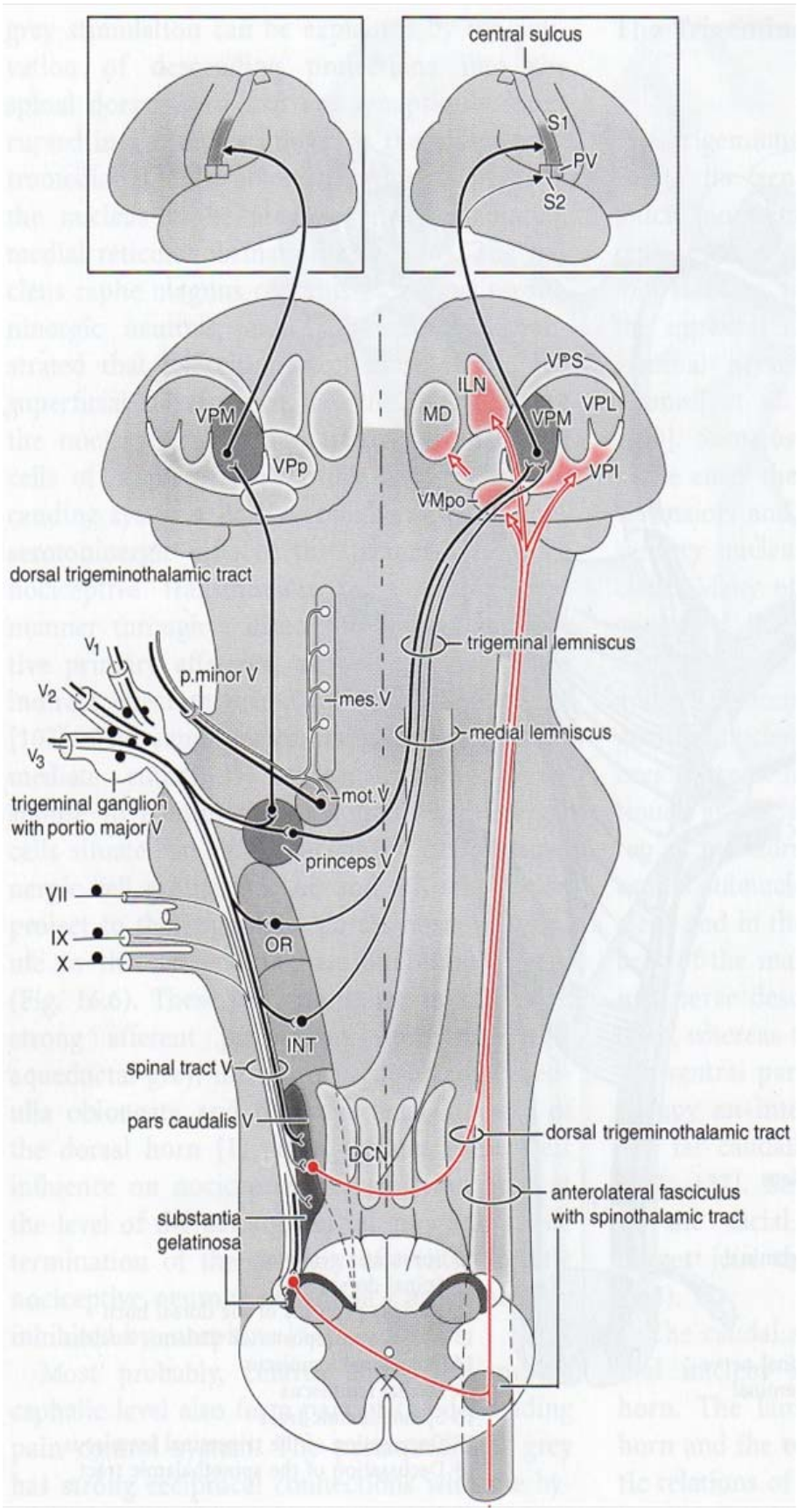
ophthalmic division occupies ventral areas of the tract (Nieuwenhuys et al., 2007). Maxillary fibres occupy an intermediate position and descend less caudally than mandibular and ophthalmic fibres, respectively (Rolls et al., 2003; Nieuwenhuys et al., 2007). Also small somatosensory components of the facial, glossopharyngeal and vagal nerve join the spinal trigeminal tract (Usunoff et al., 1997; Sessle, 2006). The pars caudalis is continuous with the dorsal horn and therefore also their laminar architecture and synaptic relations of the afferent A-δ and C-fibers are very similar (Sessle, 2002; Sessle, 2006; Nieuwenhuys et al., 2007). Efferent's from the pars caudalis cross in the caudal medulla and join the spinothalamic tract and terminate in the VMpo, MD, VPI and the ILN (Fig. 5). Projections from specific nociceptive and thermoceptive neurons in the pars caudalis lamina 1 are quite similar to their spinal counterparts and are located in the rostral VMpo and rostrally in the posterior insula (Craig et al., 1999; Brooks et al., 2002; Brooks et al., 2005).

The rostral portion of the spinal trigeminal nucleus can be subdivided into the pars oralis and interpolaris. The oralis may be included with the principal nucleus (princeps V, Fig. 5) and is connected with the facial motor nucleus, brain stem, thalamus and cerebellum (Netter and Dalley, 2000; Nieuwenhuys et al., 2007). Projections to the principal nucleus (princeps V, Fig 6) are somatotopically organized, with the face ventrally, the yaw dorsally and the intraoral cavity dorsally and caudally extending into the pars oralis (Shigenaga et al., 1986a; Shigenaga et al., 1986b; Shigenaga et al., 1990).

The entire principal sensory nucleus crosses into the medial lemniscus as trigeminal lemniscus (Fig. 5) where it terminates in the VPM. The uncrossed connections project via the dorsal trigeminothalamic tract also into the VPM. The VPM contains a bilateral representation of the intraoral cavity and processes touch, pain and temperature (Shigenaga et al., 1986a; Shigenaga et al., 1986b; Sessle, 2006; Nieuwenhuys et al., 2007).

Unlike other primary afferents, the Ia-muscle spindle afferents from the yaw muscles, (entering the brainstem in the trigeminal nerve) arise from cells within the mesencephalic nucleus (mes V, Fig. 5). The axons of these cells descend into the mesencephalic tract and then bifurcate in two branches. One of them terminates in the trigeminal motor nucleus, whereas the main branches are the Ia afferents from the masticatory muscles (not shown in Fig. 5).

Figure 6



Brainstem connections of the trigeminal nerve. Fibers from the principal sensory nucleus (princeps V), the pars oralis (OR) and interpolaris (INT) of the trigeminal's spinal nucleus decussate at the pons level for joining the medial lemniscus as trigeminal lemniscus and terminates (crossed and uncrossed) at the medial ventroposterior thalamus (VPM). VBM then projects further to SI and predominantly contra-lateral to S2.

Trigeminothalmic fibers from all layers of the pars caudalis join the spinothalamic tract and terminate in the intralaminar nuclei (ILN), posterior ventromedial nucleus (VMpo), caudal medial dorsal nucleus (MD) and inferior ventroposterior nuclei (VPI).

Axons of mesencephlic nucleus neurons (mes.V) join the motor root of the trigeminal and send branches to motorneurons innervating the yaw muscles located in the motor nucleus of the trigeminal nerve (Figure from Nieuwenhuys et al., 2007 modified by the author).

4. THE HUMAN TOOTH

In this section, basic information with respect to the human tooth as well as the periodontal apparatus in general is provided. I think, the interested reader is grateful to have some fundamental information about these two rudimentary structures.

4.1 ANATOMY

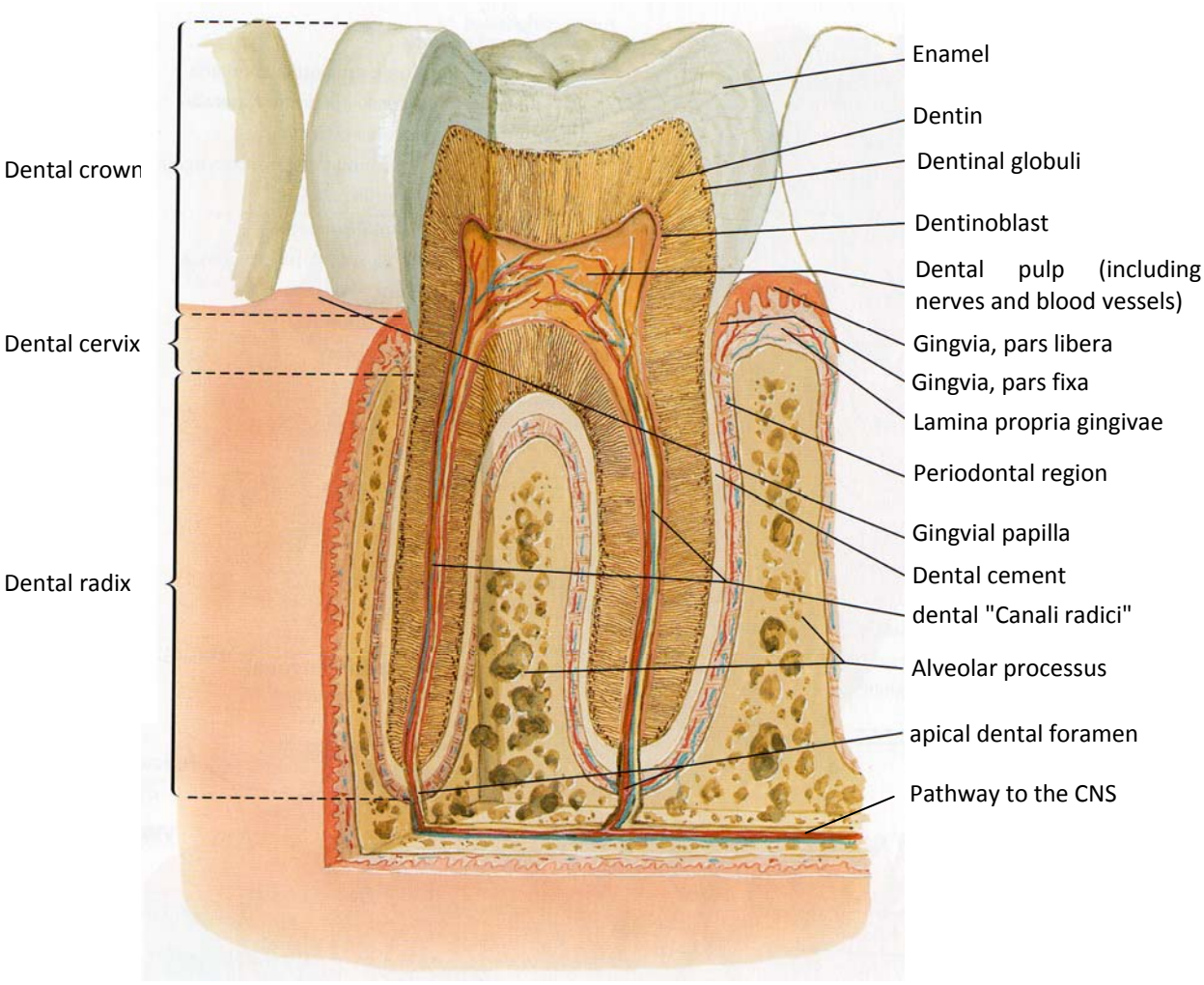


Figure 7

Illustration of the human tooth (Figure from Netter and Dalley, 2000 modified by the author).

The teeth are a unique structure. They are open-ended vital, innervated, calcified "boxes" containing dentin and cement, filled with soft neural tissue, the dental pulp and coated orally with a relatively non-vital, not innervated hard tissue, the enamel (Ash et al., 2003).

The anatomic dental crown of a tooth is the area covered in enamel and the majority of the crown is composed of dentin with the pulp chamber in the center. The anatomic root is found below the cement-enamel junction and is covered with the dental cement. As with the crown, dentin composes most of the root, which normally have pulp canals.

Humans usually have 20 primary, and 32 permanent teeth. Among the primary teeth, 10 are found in the upper (maxillar) and the other 10 in the lower (mandibular) division. They are classified as incisors, canines, and molars. In the primary set of teeth, there are two types of incisors, centrals and laterals, and two types of molars, namely first and second ones (for more details see Ash et al., 2003).

4.2 FROM THE TOOTH PULP TO A PAIN SENSATION

There is a long lasting research focus on neurobiology aspects of the tooth pulp because of its relevance to orofacial pain. The tooth has been thought as an ideal model for studying pain by virtue of its being a pure source of nociceptive input to the CNS (Sessle, 1987; McGrath et al., 1983). This concept of the exclusive pain transmitting role of the dental pulp is primarily based on uncontrolled - and often anecdotal - clinical observations as well as anatomical and electrophysiological observations that argued the way, that the dental pulp is supplied exclusively by A-δ and C-fibers (Byers, 1984; Sessle, 1988). Other sources pointed out, that teeth are a source of both, pain and low-threshold mechano-sensation, like other hard tissue (Dubner, 1986; Sessle, 1988; Ash et al., 2003). Within the tooth pulp, sensory axon-endings are predominantly of fine diameter, thus in A-δ and C-fiber range, but as (Cadden et al., 1983) showed in cats, many of these axons have rather properties of large-diameter, fast conducting A-β fibers. This observation is possibly an explanation of the phenomenon called "pre-pain", a non-painful tingling sensation evoked by electrical stimulation in human teeth (Virtanen, 1986; Virtanen et al., 1987; Ikeda and Suda, 2003). Obviously, the tooth pulp contains some A-β axons which process these clearly non-painful signals and some studies also indicate that a perception of cold or heat can be evoked by stimulating human teeth (Närhi et al., 1992; Byers and Närhi, 2000; Ikeda and Suda, 2003).

The other facet of tooth pain is the aversive characteristic people very often describe when suffering from toothache. As described in section 2.2, a pain experience contains not only the somatosensory aspect, probably more relevant are affective and emotional attributions

regarding the intensity and quality of such a pain experience. In this direction, tooth pain is ranked at the sharp end of the unpleasantness scale. It's not only tooth pain, general pain arising from the cranial region is mostly described as very tantalizing, but still, the teeth seem to have an exceptional position. A report by (Litt) substantiated these facets by pointing out that despite most dental treatments are not inherently very harmful, they are yet associated with great pain, often uncommon unpleasantness and anxiety. (Klingberg and Broberg, 2007) summarized in a review paper several aspects related with dental fear/anxiety and dental behavior management problems. They concluded that both of the facets are strongly related with pain or perceived lack of control during dental treatment. But they also found dental fear/anxiety in children from dental fear patients although they never had any bad experience towards that direction. Interestingly, such a behavior persists in approximately 5% of the children. They will have always difficulties to cooperate to dental treatment. To date, the reasons for this relatively high occurrence restricted to dental treatment are not known. Some researchers argue with biological preparedness aspects the way that the dental apparatus is inevitable for living due to its central role according to food intake (Sessle, 2002; Sessle, 2006). But both, the cranial region in general and specifically the face are additionally closely related with the emotional and affective state of a human being. And last but certainly not least; we also communicate predominantly with our face and mouth what makes it so important in our daily social environment. All these aspects together are responsible that every potentially harmful event can provoke an over-interpretation or pre-emption of what could eventuate and that’s finally a possible explanation for the special role of the face and moth when we are under pain (Litt).

The following section tries to disentangle this complicated interaction by summarizing the pertinent literature with respect to brain response circuitries due to (dental) pain.

5. NEURAL CORRELATES OF PAIN

5.1 THE PAIN MATRIX

This section provides an overview about the current state of pertinent findings with respect to the cortical network mostly observed in pain investigations. There is a bunch of literature about peripheral mediated pain processing in the human brain and specific knowledge attributes are still emerging very fast. On the other side, only three published studies examined specifically cortical activations of trigeminal mediated somatosensory related sensation and pain. Methodologically, human pain perception has been studied with different tools. Thus, EEG/MEG, PET, fMRI, pre-surgical and also post-mortem investigations led to a host of cortical and subcortical loci that were found being involved by various experimental nociceptive conditions and different clinical pain states. The experimentally related activations can be provoked by physical, chemical and psychological means.

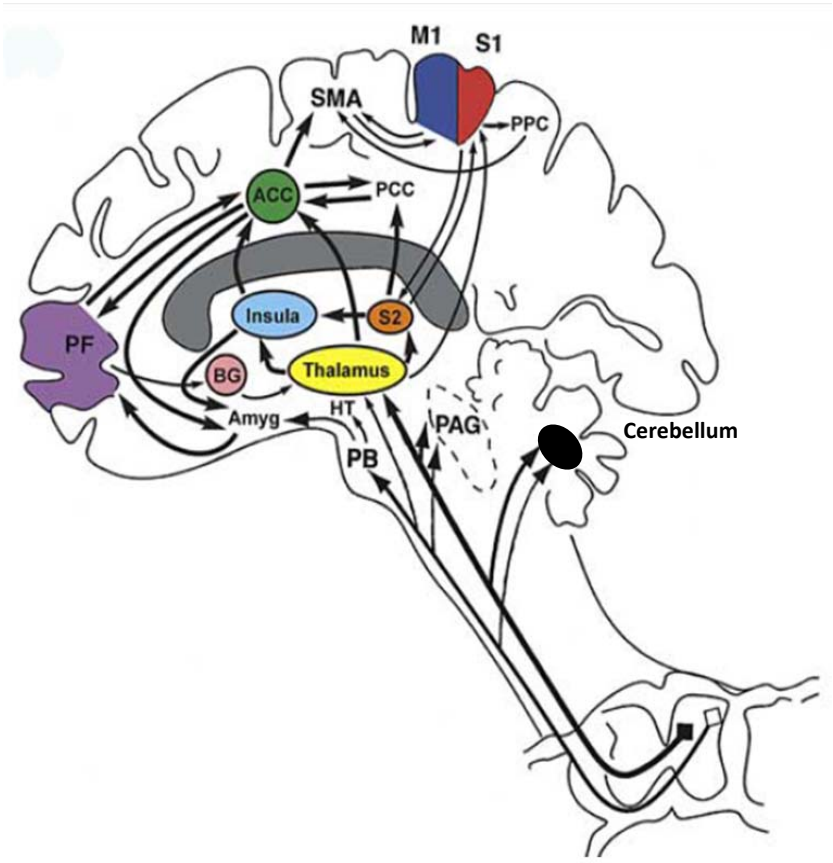


Figure 8 illustrates cortical and subcortical "core-sites" involved in human pain perception. Arrows indicate their inter-connectivity and pathways. PF=Prefrontal Cortex, BG=Basal Ganglia, ACC=Anterior Cingulate, PCC=Posterior Cingulate, SMA= Supplementary Motor Area, M1= Motor Cortex, S1/S2=Primary and Secondary Sensory Cortex, PPC=Posterior Parietal Cortex, Amyg=Amygdala,HT=Hypothalamus, PB=Parabrachial Nucleus, PAG=Periaqueductal gray (Figure from Apkarian et al., 2005 modified by the author).

Because most of the studies found a set of core sites activated, the term "pain matrix" has been suggested. Reviews of (Peyron et al., 2000; Apkarian et al., 2005; Farrell et al., 2005) and (Chen, 2008) summarize the findings of a great many of studies using different paradigms and methodologies.

The different cortical regions in figure 8 point out the current opinion in imaging related pain research, as (Casey, 2000) nicely summarized: *"pain and pain modulation is mediated, not by a simple pathway with one or a few central targets, but by a network of multiple interacting modules of neuronal activity. Simplified phrenological thinking, with complete psychological functions separate and localized, is appealing, but wildly misleading. It is far more realistic and productive to apply qualitative and quantitative spatial and temporal analyses to the distributed activity of the conscious, communicating human brain. Therefore, sensory, affective and vegetative aspects of the pain experience have to be processed in a parallel manner over cortical and subcortical structures."*

All cortical and subcortical areas depicted in figure 8 have been repeatedly demonstrated to be part of the pain matrix although the occurrences variegate tremendously considering the different investigations. For instance SI, thus the primary somatosensory cortex is activated in roughly half of all studies, whereas activity within the insular cortex is observed in almost every study (Peyron et al., 2000). Also activity within cingulate cortex and thalamus subdivisions is quite often described. Less noted but also frequently reported are cortical activations within motor-related areas like the striatum, cerebellum and supplementary motor areas as well as in regions predominantly involved in descending pain control paths such as the PAG (Peyron et al., 2000; Apkarian et al., 2005). It is also known, that some areas of the brain are specifically involved in coding for different stimulus intensities, like subdivisions of the anterior cingulate (Buchel et al., 2002; Vogt, 2005) or insular cortex (Baliki et al., 2009; Kurth et al., 2010). Other cingulate subareas (Buchel et al., 2002) are more involved in subjective processing aspects regarding affective components of a pain experience whereas aspects of aversiveness are allocated to amygdala-hippocampal circuitries (Buchel et al., 1999). Somatosensory and pain related activity within primary somatosensory areas is strongly associated with the total amount of body surface stimulated (spatial summation) and most likely also with temporal summation and attention to the stimulus (Peyron et al., 2000). Additionally, there are reports demonstrating pain related activity within frontal brain areas like dorso-lateral prefrontal (DLPFC) and medial prefrontal

(MPFC) cortex (Iadarola et al., 1998; Coghill et al., 2001). Those regions are suggested to mediate cognitive components of the pain processing system but since activity has also been observed even in the absence of an actual stimulation, it has also been partly attributed to anticipation of pain (Peyron et al., 2000; Porro et al., 2002; Porro et al., 2003).

Over all, the last decade of pain related imaging studies provide an acceptable deal of convergent data and promising results that should improve our understanding of pain processing in the human brain. There are some remaining and challenging discrepancies as well as interpretative difficulties that have to be solved in the future by conducting more sophisticated and detailed investigations.

5.2 IS THERE A DENTAL PAIN MATRIX?

As mentioned at the beginning of this chapter, only few publications existed with a rationale to study hemodynamic responses resulting from dental stimulation. Among them, Ettlin et al. (2004), investigated painless, vibrotactile dental stimulation and found predominantly activation in the supplementary motor area, the anterior insular cortex as well as lower activity around regions of the cuneus, superior occipital gyrus and post-central sulcus. However, as this work focused clearly on non-nociceptive stimulation of non pulpal-nerve origin, the results have to be carefully considered according to a possible cortical dental pain circuitry. The work of Fitzek et al. (2004) is worthwhile to be mentioned here, even if they studied not directly tooth pain, but pain mechanisms due to direct nociceptive electrical stimulation of the ophtalmic trigeminal branch. Stronger contralateral than ipsilateral activity have been observed bilateral within secondary somatosensory regions (SII), the contralateral insular cortex and the thalamus. Incorporating their single-subject analysis, in six (out of twelve) volunteers, activation within cingulate cortex subdivisions have been found. They additionally found activation within brainstem regions in three of the investigated twelve subjects. Provoking exclusively pain, Jantsch et al. (2005) demonstrated by contrasting noxious stimulation of the left central incisor and the left dorsum of the hand, that both stimulation sites tend to activate principally the same brain network but with some characteristic differences regarding the tooth. In respect of the primary somatosensory cortex (SI), they found only contralateral activation evoked by hand stimulation within the respective hand area compared to bilateral SI activation when

stimulating the tooth, respectively. Such bilaterality has not been reported for somatosensory inputs of the peripheral system in general. Considering SII and insular cortex regions, only minor differences were revealed the way, that the calculated center of gravity during hand stimulation was more medial/posterior compared to tooth stimulation. Specifically within insular subdivisions, tooth pain apparently induced stronger activation in anterior and medial parts whereas posterior parts of the anterior cingulate gyrus demonstrated stronger activity due to the hand stimulation. Differential activations were additionally found in motor and frontal brain areas including the orbital frontal cortex where tooth pain led to greater activation clusters with concomitantly higher BOLD response patterns. Further on, the effect of weak vs. strong tooth pain was compared and those areas in which tooth pain induced more activation than hand pain, significant greater activation patterns had been demonstrated what was clearly related to the more painful tooth stimuli. An interesting and unexpected result was delineated from medial frontal and right superior frontal gyri. Here, the authors observed an inverse relationship between pain intensity and BOLD response patterns, thus, the more pain a subject reported, the less activity was induced. These results were the first evidence towards the question how the brain processes pain specifically derived from a tooth. To conclude their results a basic summary can be stated the way, that an activation matrix was observable that resembled in some points the "pain matrix" known from peripheral somatosensory and pain specific investigations. But also peculiarities of tooth pain were observable. However, their study had some problematic issues because they used a mechanical and an electrical stimulus to provoke hand and tooth pain. Therefore, a direct comparability is assumable biased because of the two different stimulation modalities that make it difficult to directly compare trigeminal versus spinal nociceptive input and respective cortical activation patterns. Coming back to the title of this section; it is not clear based on the pertinent knowledge derived from the published literature whether the brain masters in a specific way nociceptive input from the trigeminal somatosensory system. There are some data pointing to a activated brain network that resembles to some degree the pain matrix, but more robust data are needed in order to elucidate the interesting and important question: is there a dental pain matrix?

6. METHODS

This section describes the stimulation method used in the conducted experiments. Most of the relevant aspects are exactly described in the respective manuscript sections; hence, I will focus here on a broader spectrum in terms of further options our setup provides. I abstain from giving theoretical basic principles of fMRI, as this topic is extensively described in specific literature.

6.1 STIMULATION DEVICE - GENERAL ASPECTS

The stimulation device applied in all studies of this thesis was originally designed to serve as hardware platform for a broad spectrum of functional electric stimulation (FES), a technique, widely used in different fields of research and clinical rationales (FES is also referred to as Functional Neuromuscular Stimulation (FNS) or Neuromuscular Electrical Stimulation (NMES)), i.e. (Blickenstorfer et al., 2008). The applications range from therapeutic interventions of muscle atrophy (Sheffler and Chae, 2007), muscle force training (Gondin et al., 2005), endurance training (Marqueste et al., 2003), pain treatment (DeSantana et al., 2008; Weiner et al., 2008), functional movement therapy (Ring and Weingarden, 2007) to restoration of functional movement skills in disabled patients using so-called neuroprostheses (Popovic et al., 2002). Even positive effects on wound healing have been demonstrated in several investigations (i.e. (Baker et al., 1997; Khalil and Merhi, 2000). The specific device - used for all studies in this thesis - has been optimized for research requirements by providing the option of free parameter programmability (Keller et al., 2002; Popovic and Keller, 2004).

6.2 THE BASIC PRINCIPLES OF FES

As aforementioned, there is a broad spectrum according to the application field of FES. However, the fundamental principles are very similar. Low levels of electric current are administered to stimulate physical or bodily functions, by either stimulate or suppress specific parts of the nervous system. FES is applied to peripheral nerves that control muscles

or muscle groups either through self adhesive surface electrodes, needles or implanted electrodes, which then is termed transcutaneous electrical stimulation (TES). The electric pulses are applied between pairs of electrodes. Ion currents are driven between the positive electrode (anode) and the negative electrode (cathode). At the anode, positive ions in the underlying skin are repelled and negative charged ions are simultaneously attracted. The cathode acts in the opposite way, resulting in a current flux of ions, defined as the movement of charged particles (Baker, et al. 1993). Since the electric current seeks the way of least resistance it will flow more easily through tissue with low impedance. Thus, due to the high impedance of the skin with its horny layer, the electric current rather passes through the underlying structures. Most of the stimulating current therefore bypasses the nerve fiber and flows through the extracellular fluid which has lower impedance. Because of this, only a relatively small fraction of the electrical current is able to pass through the membrane of an axon. The relationship between the diameter of an axon and its resistance to longitudinal flow (along the axon's axis) is reciprocal the way, the larger the axon diameter, the lower is the resistance for the current according to pass the membrane. Thus, larger nerve axons have lower current thresholds and are therefore more easily exited by peripherally applied electrical stimuli (Baker, et al. 1993).

6.3 HOW TO INDUCE TOOTH PAIN?

As mentioned in the preface section, this was a challenging topic that took us some time but after several failure attempts, we finally developed a reliable setup more or less easy to construct and handle. For this, maxillary alginate impressions were taken from the subjects' dentitions for fabrication of soft dental acrylic splints. The same procedure is also applicable for the mandible dentition, so basically, we are not limited to certain teeth. Then, pairs of stainless steel electrodes were embedded in each individual dental splint opposite the labial and palatal surface center of the target teeth. In order to minimize the impedance between tooth and corresponded electrode, we applied a specific hydrogel (AMGEL Technologies, AG602-6, 8520 Lystrup, Denmark) on each electrode and covered it with a thin layer of toothpaste (Signal Microgranuli, Unilever, Zug, Switzerland). To avoid radiofrequency contamination of the stimulation current, specially shielded wires were used.

The nociceptive stimulation was then performed by means of the Compex Motion System (Keller et al., 2002) and the experimental protocol was controlled by the Presentation software via the systems parallel port using a self made interface. Meanwhile, several steps of this initial approach have undergone further development, always with the rationale to increase the applicability and effectiveness of the stimulation setup.

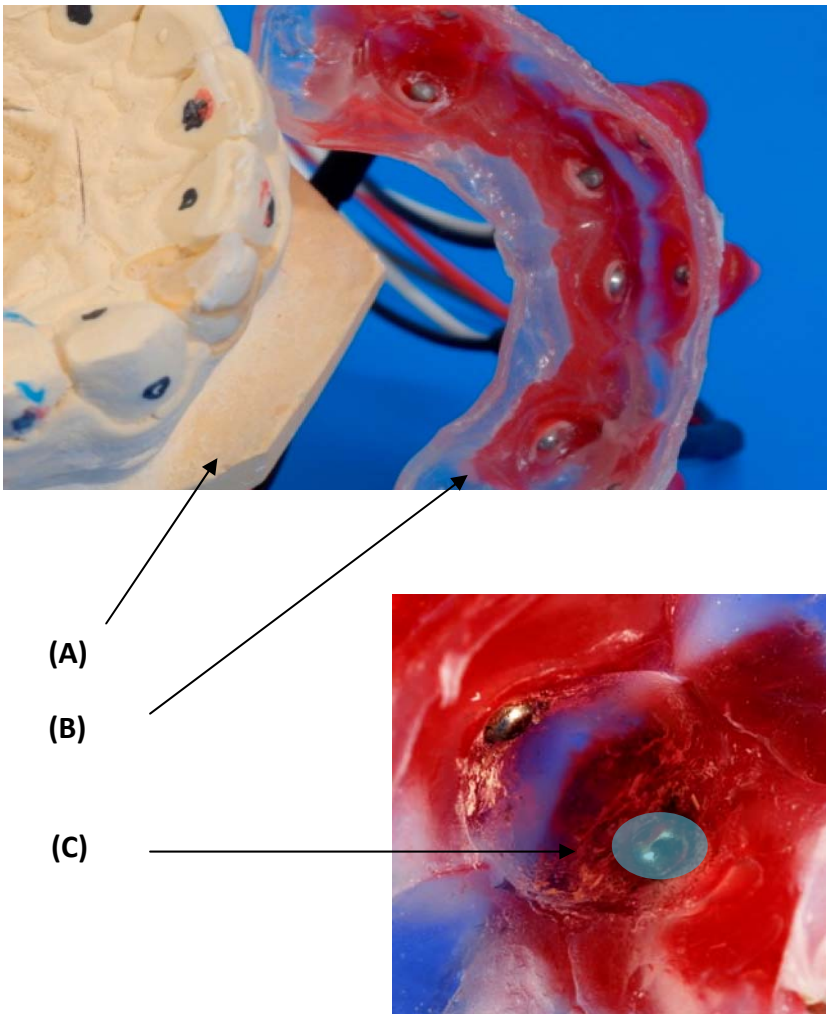


Figure 9 illustrates the basic components of the stimulus application device with the alginate imprint (A) and the individual constructed acrylic splint (B), here with 4 stainless steel electrodes embedded.

The amount of steel electrodes is principally free, theoretically, all teeth could be stimulated. There is a limitation factor in the fMRI as with increasing electrodes, the disturbances within the magnetic field also increases. With 4 pairs of electrodes, we had only marginal disturbances which can be disregarded.

To increase the current flow, a special hydrogel was applied on the electrodes (showed for one electrode in (C)), which then was covered by a thin layer of toothpaste, immediately before subjects administered the splint.

The stimulation parameters were chosen in accordance to conform as precise as possible the descriptions of the sensations that patients report who are suffering from dentinal hypersensitivity. They often define their sensations as short, sharp and fulgurant pain. After several (mostly painful) self-attempts, we found the stimulation parameters, which resemble the described characteristics very well and - really important - does not harm the dental apparatus in any way. To reach this demanding and critical aim, we used a biphasic and bipolar current with 1ms duration and 100 Hz frequency. With these characteristics, the pulp fibers should be depolarized, leading to a generation of an action potential and due to the biphasic and bipolar form, the applied charge should leave the body without summing up because the secondary pulse of the biphasic wave balances the applied charge of the primary pulse (Popovic and Keller, 2004; Peckham and Knutson, 2005).

For optimizing purpose, we conducted an experiment with 10 subjects to find out, at which moment after inserting of the acrylic splint, the conductivity between tooth and electrode allows for an optimal stimulus application. Thus, we measured the impedance between tooth and electrodes at three different time-points, namely, (i) directly after inserting the device, (ii) after 10 minutes and (iii) after 40 minutes. We found a significant impedance decrease when comparing (i) and (ii) and (i) and (iii), but not when comparing (ii) with (iii). Hence, with this experiment, the ideal moment of the stimulation onset has been determined from 10 minutes after a subject had inserted the splint.

The following sections contain the empirical work of this thesis. All of them were conducted in order to investigate specific attributes of brain processes due to experimentally induced dental sensations ranging from painless - but clearly perceivable - to painful. Specific aspects regarding intensity coding, somatotopic localization, lateralization and cognitive processing of the perceived sensations have been distinctively addressed.

7. EMPIRICAL STUDIES

7.1 STUDY 1

Interindividual differences in the perception of dental stimulation and related brain activity

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Published in:

European Journal of Oral Sciences

ABSTRACT

For identical diagnoses in the trigeminal innervation territory, individual differences can be clinically observed among reported symptoms, such as dysesthesia and pain. Different subjective perceptions of unpleasantness and pain intensity may have different cortical substrates. The aim of this study was to identify brain areas in which activation depends on the subjective perception (intensity and unpleasantness) of electric dental stimulation. Electric stimuli with increasing intensity were applied to maxillary canines in 14 healthy volunteers. Ratings for stimulus intensity and unpleasantness perceived across the stimulation session were reported post-scan on 11-point numerical scales. The rating values were then included as covariates in the fMRI group analysis. Interindividual differences of intensity ratings were reflected in differential activity of the following brain areas: superior parietal lobule, superior temporal gyrus/anterior insula, inferior and middle temporal gyrus, lingual gyrus, anterior cingulate and caudate nucleus. Differences related to unpleasantness ratings were reflected in the lingual gyrus. In conclusion, differences of perceived intensity between individuals are reflected in differential activity of a set of brain areas distinct from those regions reflecting rating differences of unpleasantness.

Keywords: tooth; electric stimulation; sensation and perception; magnetic resonance imaging; toothache model

INTRODUCTION

Orofacial sensory disorders are multidimensional subjective experiences and patients may report trigeminal symptoms ranging from unpleasant sensations (dysesthesia) to pain (Canavan et al., 1994; Hillerup, 2007; Clark and Ram, 2008). Multidimensionality and considerable interindividual perceptive variability complicate not only clinical assessment and management, but also experimental investigations. Evidence put forward reveals involvement of a complex brain network representing sensory-discriminative, affective-motivational and cognitive-evaluative aspects of unpleasant sensations (Melzack and Casey, 1968). Some researchers therefore advocate separate assessment of the two pain dimensions intensity and unpleasantness for experimental pain studies (Price et al., 1987).

Neuroimaging methods nowadays enable to correlate neurophysiologic reactions and subjective perception in response to experimental stimuli. Distinct central pathways and brain regions have been described to be involved in the differential processing of pain intensity and unpleasantness (Price, 2002). Multiple factors such as age, gender, sociodemographic factors, personality, and socialization experiences have been implicated in influencing stimulus appraisal (Lazarus and Folkman, 1984). A multitude of imaging studies (EEG, EMG, PET and fMRI) investigated spinally induced sensations of different modalities and intensities (e.g. Peyron et al., 2000; Farrell et al., 2005).

However, little information is available on cortical aspects of interindividual perceptive variability of spinal sensations and pain (Coghill et al., 2003). Regarding sensations of dental origin, only few studies report on neural correlates of tooth sensations and toothache (Ettlin et al.,2004; Jantsch et al., 2005), possibly because of technical challenges related to the stimulation setup.

Electric stimulation of human teeth has been widely used in the past. Azerad and Woda (1977) implanted two steelwires directly into the dental pulp and stimulated with monophasic square pulses. The stimulated third molar teeth were extracted subsequently. A less invasive method was developed by (Kempainnen et al., 1985). Dental pain was administered with a constant current tooth stimulator. They applied the cathode to an intact upper incisor and the anode to the arm of the subjects. This method was recently modified for MRI compatibility by using a carbon wire lead between the tooth electrode and the stimulus amplifier and by relocating the anode from the arm to the leg (Jantsch et al., 2005). This method requires that the cathode be glued to the stimulated tooth, which may be quite time consuming, especially for experiments involving stimulation of multiple teeth.

The Compex Motion stimulator is a portable electric stimulation device successfully used for a wide range of transcutaneous functional electric stimulation, including therapeutic applications (Popovic, 2006). It has not been previously used for dental stimulation.

The aim of this study was to identify with the Compex Motion stimulation device neural correlates of subjective differences regarding intensity and unpleasantness ratings of dental sensations.

MATERIALS AND METHODS

17 healthy volunteers entered the study (7 female, 10 male, age 26 – 45). Inclusion criteria required test teeth to be caries free, vital, and without attachment loss. Usual MRI contra-indications led to exclusion (Ettlin et al., 2004). Subjects received detailed information about the experimental procedure, provided written informed consent according to the approval by the local ethics committee. The study was conducted according to the guidelines of the Declaration of Helsinki for treatment of experimental human subjects.

The Compex Motion stimulator was used for electric stimulus generation. It was controlled by a chip card, which was programmed by custom made software capable of generating any arbitrary stimulation sequence (Keller et al., 2002). The stimulation sequences were stored on memory chip-cards. Using biphasic pulse forms of 1 ms duration, a left or right maxillary canine was stimulated with a pair of stainless steel electrodes, mounted on a custom made acrylic dental splint (Fig. 1 and 2). In order to minimize electric resistance, a punched round piece of hydrogel (AMGEL Technologies Europe (Type: AG 602 Sensing gel), 8520 Lystrup, Denmark) with 3 mm diameter was placed on anode and cathode and was covered with a thin layer of toothpaste (Signal Microgranuli, Unilever, Zug, Switzerland). Phantom MRI measurements performed prior to subject testing did not reveal any disturbance of the MR signal with this setup. 11-point numerical scales were employed for the post-scan ratings of perception intensity (ranging from "not perceptible" to "clearly perceptible") and unpleasantness (ranging from "not unpleasant at all" to "maximal unpleasant").

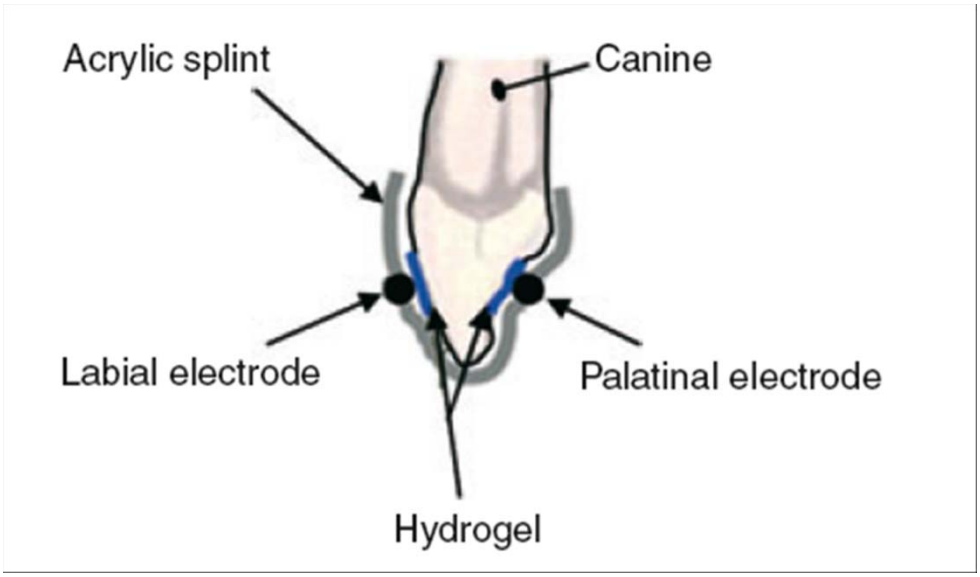

Figure 1

Schematic illustration of the splint with embedded stimulation electrodes and the placements of the hydrogel.

Figure 2

Photograph of the individual fabricated acrylic splint.

EXPERIMENTAL PROTOCOL

Prior to the fMRI protocol, we tested in different subjects if electric dental stimulation with the Compex Motion system was tolerable and assessed possible perception differences related to side of stimulation. Left and right canines were stimulated in seven subjects during pretrial testing. As no significant differences were observed (Table 1; paired t-test, CPT left vs. right $p = 1$; PPT left vs. right $p = 0.82$), and to reduce scan time as well as volunteer distress, we determined to stimulate only one canine in the MR scanner. Half of subjects were stimulated on the right and the other half on the left side.

Subjects were comfortably placed on the scanner table in supine position with the acrylic splints inserted for 10 minutes prior to starting the protocol. Subjects were instructed to maintain eyes closed during the trials. After acquisition of one anatomical image, electric stimuli (1 ms pulse width) with increasing intensity were applied to one randomly selected maxillary canine with inter-stimulus intervals randomized between 7 and 10 sec. The first stimulus intensity was set at 0.6 mA, with stepwise increases of 0.4 mA for each stimulus up to the inherent upper system limit of 25 mA (62 trials totally). Subjects indicated their respective thresholds by pressing an alarm button once for current perception threshold (CPT) and twice for pain perception threshold (PPT) (Fig. 3). Because of current output limitation (25mA), none of the subjects reached pain tolerance which would have led to abortion of the run.

After exiting the scanner, subjects were asked to attribute their sensory perceptions to dental and/or mucosal tissue and then asked to evaluate the overall intensity and unpleasantness experienced during the procedure on 11-point numeric rating scales (Table 2). We additionally asked for several qualitative aspects and some specific characteristics of the induced sensation by a questionnaire implemented in a half open interview.

Table 1

During a training session, both maxillary canines were stimulated in seven subjects to assess possible side differences for CPT and PPT. Mean = mean value; SD = standard deviation.

Subject	Tooth	
	Right canine CPT/PPT [mA]	Left canine CPT/PPT/ [mA]
1	4.2 / 13	4.2 / 12.6
2	3.4 / 15.4	3 / 14.6
3	5.4 / 18.2	5.0 / 18.6
4	1.4 / 6.6	1.8 / 5.8
5	2.2 / 9	1.8 / 9.8
6	1 / 10.2	2.2 / 10.6
7	5.8 / 13	5.4 / 13
Mean	3.34 / 12.2	3.34 / 12.14
SD	1.90 / 3.94	1.52 / 4.02

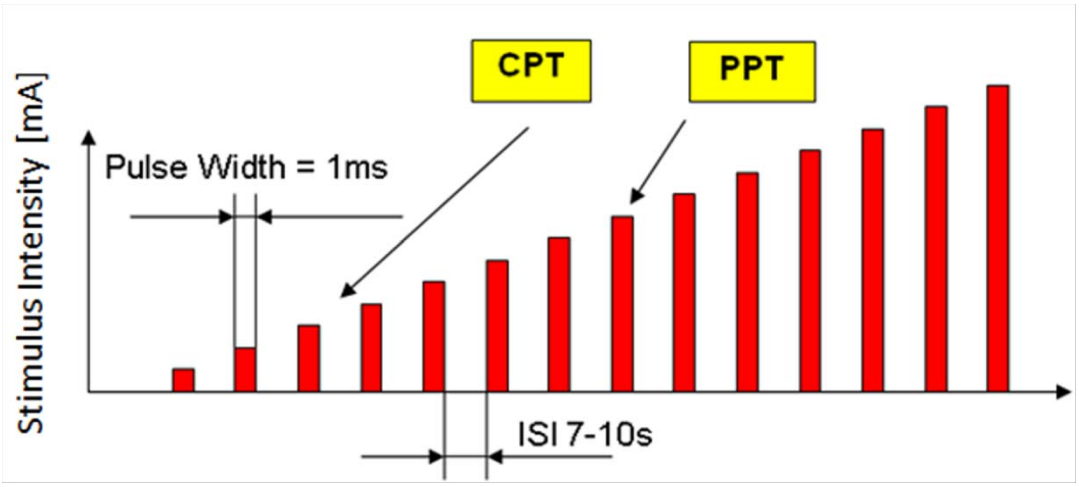

Figure 3

Stimulation protocol depicted with relative scale dimensions. Beginning at 0.6 mA, increments 0.4 mA, maximal 25 mA and 62 stimuli. Also illustrated are the (theoretical) current perception threshold (CPT) and the pain perception threshold (PPT). ISI = interstimulus interval.

FMRI DATA ACQUISITION

fMRI measurements were performed on a Philips 3.0 T Achieva system (Philips Medical Systems, Best, The Netherlands). For the functional scans, a blood oxygen level dependent (BOLD) sensitive single-shot gradient echo planar imaging sequence was used with 33 axial slices, covering the entire cerebrum, using a 8 channel receive only head coil. Parameters: echo time = 30 ms, flip angle = 75°, repetition time = 2500 ms, slice thickness = 4 mm, inter-slice gap = 0 mm, field of view = 230 mm and matrix size in plane = 128 x 128, voxel size of 1.72 x 1.72 x 4 mm^3. The first three scans were acquired to reach steady state magnetization and then discarded. 180 high-resolution T1 weighted axial images (spoiled gradient echo) were acquired with TR = 20ms, flip angle = 20°, voxel size = 0.98 x 0.98 x 1.02 mm^3, FOV = 24 cm, matrix = 256 x 192, which were used as an underlay for individual pre-analyzing functional maps.

STATISTICAL ANALYSIS AND IMAGING DATA PROCESSING

Group means and standard deviations of the CPT and PPT as well as the subjective ratings were determined and consistency of these variables over subjects was assessed by calculating the coefficient of variation (CV) (Frank and Althoen, 1995). In order to compare possible differences according to the “side of stimulation”, a one-way ANOVA with "side of stimulation" as factor and “CPT”, “PPT”, “overall unpleasantness” and “intensity” ratings as dependent variables was performed. Another one-way ANOVA was performed in order to compare differences between female and male with "gender" as factor. SPSS for Windows (Release 14.0.0) was used for statistics.

Functional image analysis was done using SPM5 (http://www.fil.ion.ucl.ac.uk/spm) software package running on MatLab R2006b (Mathworks, Natiek, MA, USA). In a first step, spatial realignment and reslicing of the whole series to the first image as reference was performed. Detected movement did not exceed 1.5 mm (translational) and 1° (rotational) compared to the first image in the time series. For single subject analysis, data were realigned, normalized to the Montreal Neurological Institute (MNI) template, spatially smoothed with a Gaussian Kernel of 6 mm (FWHM) and scaled to the global mean.

First, for each subject a generalized linear model (GLM) was set-up including regressors coding the increasing stimuli and enabling comparison of stimulation events with baseline by application of linear contrasts. This resulted in beta values showing the size of effect of the

stimulus on the BOLD signal (Friston et al., 1997). Thereafter, for the group analysis, a random-effect GLM was set up which enabled correlation of the beta values with the individuals' ratings of overall pain intensity and unpleasantness, respectively. This allowed identifying brain areas that might explain interindividual differences in somatosensory intensity and unpleasantness ratings.

RESULTS

BEHAVIORAL DATA, VERBAL REPORTS

All subjects located sensory perception in the stimulated tooth only. Every subject reached CPT and PPT. Each subject also reported the stimulus application as increasingly perceived. We encountered technical difficulties with MR image reconstruction in three subjects and therefore 14 datasets were considered for further analysis.

THRESHOLDS AND OVERALL PERCEPTION RATINGS (TABLE 2)

The one-way ANOVA brought no differences according to the “side of stimulation” factor for CPT ($F = 0.731$, $p = 0.409$, mean square error (MSE) = 7.566, df = 12), PPT ($F = 0.154$, $p = 0.701$, MSE= 39.223, df = 12), intensity ($F = 0.000$, $p = 1.00$, MSE= 5.952, df = 12) and unpleasantness ($F = 0.044$, $p = 0.838$, MSE= 6.548, df = 12). Also according to the "gender" factor, no significant effects were observed for CPT ($F = 0.747$, $p = 0.404$, MSE= 7.556, df = 12), intensity ($F = 1.120$, $p = 0.311$, MSE= 5.444, df = 12) and unpleasantness ($F = 0.023$, $p = 0.883$, MSE= 6.559, df = 1), whereas a trend in PPT ($F = 3.495$, $p = 0.086$, MSE= 30.767, df = 12) appeared.

CPT on average was observed at a stimulus intensity of 2.6 mA (SD ± 2.7) and PPT at 16.0 mA (SD ± 6.1). Ratings for overall intensity ranged from 1 to 8 (mean = 4.57, SD ± 2.34, CV = 0.51), and for unpleasantness from 2 to 10 (mean = 6.29, SD ± 2.46, CV = 0.4). Both, CPT and PPT were important to assure, that the subjects perceived the stimulation comparable, but are not of further particular interest for the objective of the study.

Table 2

Thresholds values for current perception and pain perception as well as post-hoc 11-point numeric scale ratings for intensity and unpleasantness.

M = male, F = female; r = right canine, l = left canine

Subject / sex	Side of Stimulation	CPT (mA)	PPT (mA)	Overall Rating	
				Intensity	Unpleasantness
1 / M	r	4.2	19.8	5	8
2 / F	l	0.6	15.8	6	5
3 / M	l	4.2	15.8	3	8
4 / M	r	10.2	24.6	4	6
5/ M	r	0.6	25	1	3
6 / F	l	3.0	13.8	4	6
7 / F	r	0.6	5.4	4	3
8 / M	l	0.6	10.6	8	10
9 / M	l	1.4	25	1	2
10 / F	r	0.6	19.4	8	10
11 / M	l	3.4	13.4	3	6
12 / F	r	1.4	13	3	6
13 / F	r	5.0	9.4	7	7
14 / M	l	0.6	13	7	8

IMAGING DATA

The highest BOLD effect size was found in the cerebellum(posterior lobe, bilateral), with a luster size of 25 voxels on the right side and 37 voxels on the left side, respectively. The argest cluster (65 voxels) was identified at the junction of the superior temporal gyrus, anterior insula, and inferior frontal cortex, followed by the lingual gyrus, the middle frontal gyrus, the anterior cingulate cortex (ACC), the precuneus, and the inferior frontal gyrus (Table 3).

Correlation of the postscan pain-intensity rating with the BOLD effect size revealed peak voxel activity in the superior parietal lobule. The strongest activation was localized at the junction of the superior temporal gyrus, anterior insula, and inferior temporal gyrus, cerebellum, middle temporal gyrus (data not shown), lingual gyrus, ACC, and caudate nucleus (Table 3, Fig. 4). Of all regions described in Table 3, only the lingual gyrus BOLD effect size significantly correlated with the subjective evaluation of unpleasantness (Table 3, Fig. 5).

Table 3

Significant activated regions of the random effect analysis without considering the overall ratings, with the associated intensity ratings and the unpleasantness ratings. All results are uncorrected for multiple comparisons with p = 0.0001 and corrected according to the cluster thresholds of p < 0.05.

Anatomical description	MNI coordinate			
	X / Y / Z	max t-value	cluster size	cluster p
Without rating				
Cerebellum Posterior Lobe (right)	26 -74 -28	9.41	25	0.002
Cerebellum Posterior Lobe (left)	-16 -80 -36	9.38	37	0
Superior Temporal Gyrus Anterior Insula	-48 16 -6	8.47	65	0
Lingual Gyrus	0 -74 -2	7.99	15	0.026
Middle Frontal Gyrus	42 38 28	7.46	15	0.026
Anterior Cingulate	0 24 26	7.07	30	0.001
Precuneus	2 -48 44	6.8	14	0.034
Lingual Gyrus	6 -86 -8	6.8	16	0.02
Inferior Frontal Gyrus	-44 8 26	6.22	14	0.034
Intensity rating				
Superior Parietal Lobule	-26 -68 52	10.21	14	0.034
Superior Temporal Gyrus Anterior Insula	-48 8 -10	9.7	73	0
Inferior Temporal Gyrus	62 -54 -8	9.63	19	0.009
Cerebellum Posterior Lobe (right)	26 -74 -28	9.14	22	0.004
Cerebellum Posterior Lobe (left)	-16 -80 -36	8.75	19	0.009
Lingual Gyrus	0 -74 -2	8.33	16	0.02
Anterior Cingulate	0 24 26	7.93	23	0.003
Caudate nucleus	16 0 14	7.7	17	0.015
Unpleasantness rating				
Lingual Gyrus	8 -84 -8	7.21	13	0.046

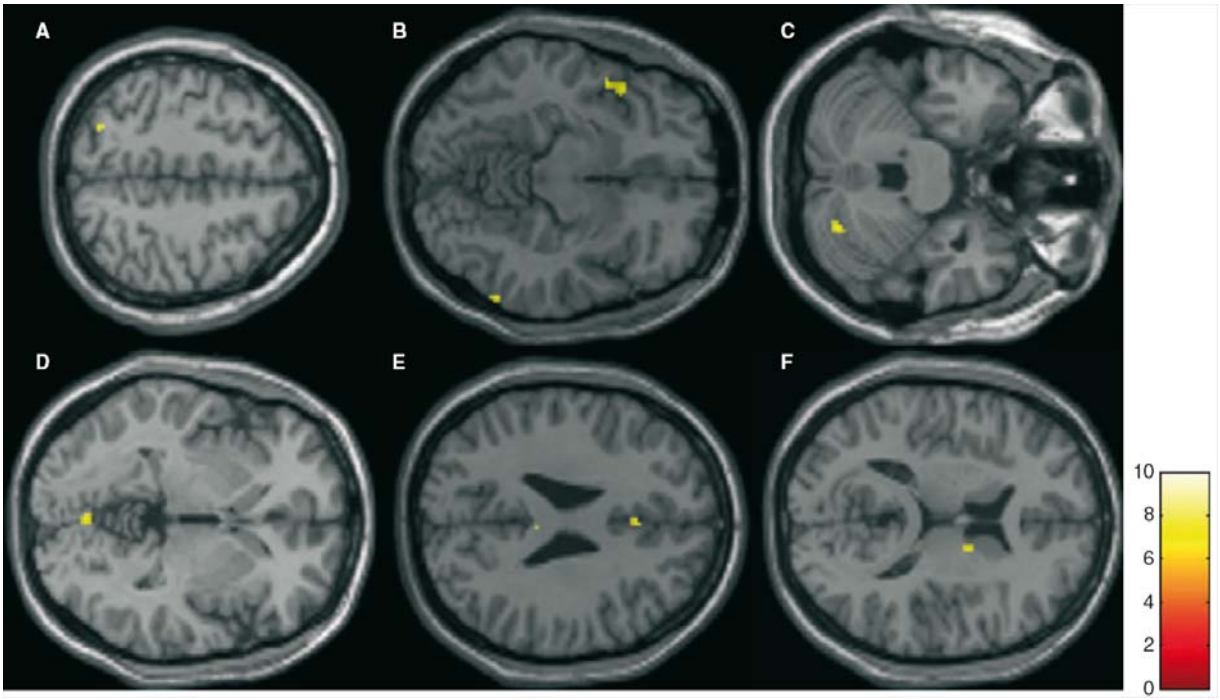

Figure 4

BOLD responses associated with interindividual differences in intensity ratings overlaid on the standard MNI template brain. Listed are clusters of at least 10 neighboring voxels ($p = 0.0001$, uncorrected for multiple comparisons) which survive a corrected cluster threshold of $p < 0.05$. a). Superior parietal lobule, b) superior temporal gyrus, anterior insula and inferior temporal gyrus, c) the cerebellum, d) lingual gyrus, e) anterior cingulate, f) caudate nucleus.

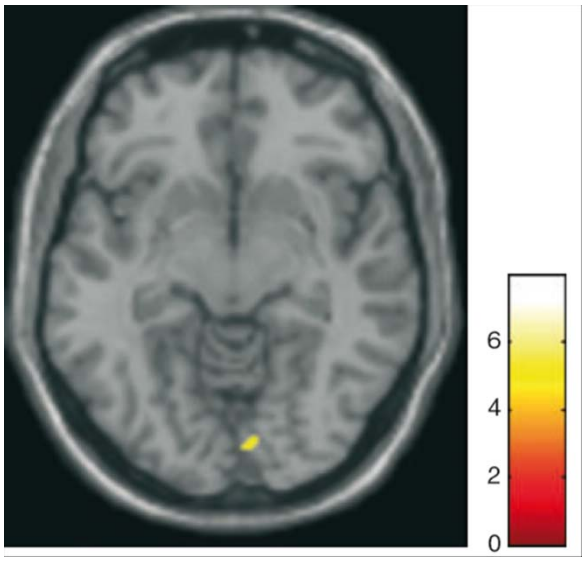

Figure 5

BOLD responses associated with interindividual differences in unpleasantness ratings overlaid on the standard MNI template brain. Listed are clusters (lingual gyrus) of at least 10 neighboring voxels ($p = 0.0001$, uncorrected for multiple comparisons) which survive a corrected cluster threshold of $p < 0.05$.

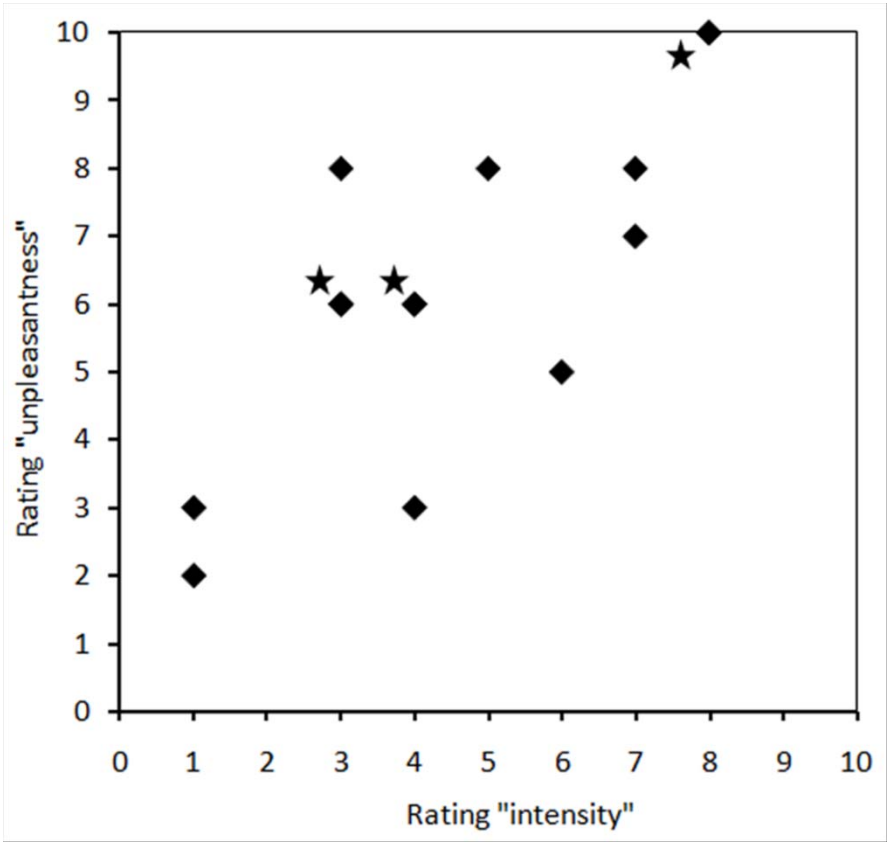

Figure 6

Correlation between intensity and unpleasantness ratings (paired samples correlation: r = 0.77, p = 0.001). Star label (*) indicates that measurement values of two subjects coincided.

DISCUSSION

fMRI studies on cortical activation related to dental stimulation are few, possibly due to technical challenges (Ettlin et al., 2004; Jantsch et al., 2005). The current study reveals that it is possible within a 3 T fMRI setting to apply electrical stimuli to healthy human canines and thus to induce stimulus perceptions ranging from innocuous sensations to pain. Our main findings are that post-hoc ratings for intensity and unpleasantness perceptions are reflected in activations of different brain regions (indicating different interpretation of unpleasantness and intensity). Similar findings were reported for thermal stimuli applied to the volar forearm (Wagner et al., 2001). We additionally found that activation in some areas described to be part of the cortical pain matrix significantly differed between subjects. Post-scan

intensity ratings of electrically evoked dental sensations correlated with differential activity in a subset of brain areas related to unpleasantness ratings.

Methodological limitations are the lack of “on-line” rating of sensations (i.e. rating during MRI scanning), which will be discussed in more detail below. Another limitation is the absence of a control scenario for potential cross modality effects. Such effects have been unexpected in this study and should be addressed in upcoming investigations. Third, we have included both just perceptible and painful stimuli in our BOLD analyses. This makes it impossible to relate our results clearly to either painful of painless dental stimulation.

A notable difference to previous findings (Jantsch et al., 2005) was that in the present study, only a subthreshold trend of a BOLD signal increase was detected in either somatosensory cortices (SI and SII, not shown). This can be explained by our approach of including all stimuli from barely to distinctly perceived. Nonetheless, we found that by increasing statistical sensitivity through reduction of the search area to the classical pain matrix areas, SI indeed revealed a significant BOLD signal increase. Furthermore, experimentally induced activation changes in lingual gyrus are somewhat unexpected, since this region is functionally known as a secondary visual area. However, it has been shown that analgetic opioids modulate activity in the lingual gyrus, among other areas (Wagner et al., 2001; Willoch et al., 2003). It was shown that lingual gyrus activity increases when a visual stimulus is accompanied by a tactile stimulus as compared with a visual stimulus alone (Macaluso et al., 2000) or other crossmodal combinations of stimuli (Calvert, 2001). We hypothesize that the unfamiliar dental stimulation acts as a potent crossmodal stimulus, involuntarily drawing attention. Although not controlled for, visual processing may have been influenced by the electric stimuli despite subjects were asked to close their eyes during the scanning procedure. In view of our results, it seems worthwhile to control or experimentally manipulate visual stimulation during upcoming experiments to further elucidate this interaction.

We were interested in whether objective neural correlates can be identified related to subjective differences regarding intensity and unpleasantness ratings of electrically evoked dental sensations. We opted to defer the evaluation until after the MRI session, which is not uncommon for experimental pain protocols (Coghill et al., 2003; Tracey et al., 2002; Bantick et al., 2002; Valet et al., 2004; Seminowicz et al., 2004). This approach is clearly a study limitation, since it is less suitable to describe the psychophysics of stimulus perception compared to online rating. The latter, however, has been shown to influence cortical

activation. Specifically, enhanced BOLD signals may be linked to closer stimulus evaluation and attention focusing (Schoedel et al., 2008). Post hoc ratings on the other hand may likely be influenced by memory effects (Price et al., 1999), or personality factors, e.g. trait anxiety and coping styles (Lazarus and Folkman, 1984).

Current study revealed that among individuals who differently report unpleasantness and intensity of electrical dental stimulation, subjective ratings are related to brain activation in distinct cortical areas. It thus confirms psychophysical evidence demonstrating separate influences of these two dimensions. On cortical levels, the investigation of Coghill revealed more frequent and robust activation in the ACC, SI and prefrontal cortex in subjects who were more sensitive to pain compared to less sensitive subjects (Coghill et al., 2003).

Our data provide a rather high coefficient of variation for post hoc ratings of pain intensity (mean 4.57, SD ± 2.34; CV: 0.51), suggesting individual differences regarding pain appraisal of electrically evoked dental sensations. This fact significantly correlates with the BOLD effect size in several areas, namely the superior parietal lobule (SPL), the junction between anterior temporal pole, anterior insula and inferior frontal gyrus, the superior temporal cortex, cerebellum, lingual gyrus, ACC and caudate nucleus. As the activation characteristic of the ACC can be interpreted as concordant with findings reported by Coghill (2003), we support their suggestion that the ACC is likely one of the crucial components in the evaluation of subjectively perceived stimulus intensity. Thus, intersubject variability of the BOLD signal in these areas should be interpreted cautiously, since it is hard to interpret a change in effect size if this effect has no significance with regard to the experimental condition investigated. However, the BOLD signal in areas involved in the processing of dental nociception is correlated with the retrospective appraisal of the intensity experience: anterior insular region, posterior cerebellar regions, the lingual gyrus and the ACC (Fig. 4 and 5). Lingual gyrus activity may denote noxious influence on the attentional system (Macaluso et al., 2000; Tracey et al., 2002) (Fig. 5). ACC has been shown to encode pain intensity (Coghill et al., 2003; Büchel et al., 2002) and we also found stronger ACC activity in subjects who assessed the protocol as more intense.

Based on previous studies applying thermal cutaneous stimulation paradigms, we expected that unpleasantness ratings co-vary with neural activity in the ACC (Rainville et al., 1997; Tölle et al., 1999). In contrast, the present study revealed lingual gyrus activity modulation related to the experience of unpleasantness evoked by electrical dental stimulation. This

finding may indicate crossmodal attentional modulation of the visual system. The relationship between the hemodynamic response in the lingual gyrus and increased unpleasantness can be understood by higher distress and resulting modulation of other sensory modalities (*e.g.* the visual system). Furthermore, novelty of stimulus location and type of stimulus, individual arousal, visceral and somatomotor responses all contribute to subjective perception of unpleasantness, reflecting our perception of pain as intrusive for both body and consciousness. For Damasio, "the essence of feeling an emotion is the experience of such changes in juxtaposition to the mental images that initiated the cycle" (Damasio, 1994). Introspection as a mean of assessing a conscious experience may be linked to the visual system (lingual gyrus activity).

In conclusion, this pilot study revealed that BOLD signals in some of the cortical areas involved in processing intensity co-varies with the subject's rating of intensity, confirming previous findings related to stimulus application in other body areas. Unexpectedly, unpleasantness ratings did not co-vary with ACC activity modulation, but rather with changes in lingual gyrus activity, possibly indicating crossmodal attentional modulation.

This paradigm could be further developed towards a valid model of experimentally induced toothache and thus may shed light on cortical processing of acute and chronic dental pain.

ACKNOWLEDGMENTS

This work was supported by *GlaxoSmithKline*, Consumer Healthcare, Weybridge, UK, the *Swiss Dental Association* and the *Swiss Society for Endodontology.*

REFERENCES

Canavan D, Graff-Ratford SB, Gratt BM. Traumatic dysesthesia of the trigeminal nerve. *J Orofac Pain* 1994; 8: 391-396.

Hillerup S. Iatrogenic injury to oral branches of the trigeminal nerve: records of 449 cases. *Clin Oral Invest* 2007; 11: 133–142.

Clark GT, Ram S. Orofacial pain and neurosensory disorders and dysfunction in cancer patients. *Dent Clin North Am* 2008; 52: 183-202

Melzack R, Casey KL. Sensory, motivational and central determinants of pain. In: KENSHALO DR, ed. The Skin Senses. Charles C. Thomas, Springfield, 1968; 423–439.

Price DD, Harkins SW, Baker C. Sensory-affective relationships among different types of clinical and experimental pain. *Pain* 1987; 28: 297–307.

Price DD. Central neural mechanisms that interrelate sensory and affective dimensions of pain. *Molecular interventions* 2002; 2: 392-403.

Lazarus RS, Folkman S. Stress, Appraisal, and Coping. Springer Publishing Company, Heidelberg 1984.

Peyron R, Laurent B, Garcia-Larrea L. Functional imaging of brain responses to pain. A review and meta-analysis. *Neurophysiol Clin* 2000; 30: 263-288.

Farrell MJ, Laird AR, Egan GF. Brain activity associated with painfully hot stimuli applied to the upper limb: a meta-analysis. *Hum Brain Mapp* 2005; 25: 129-139.

Coghill RC, Mchaffie JG, Yen YF. Neural correlates of interindividual differences in the subjective experience of pain. *Proc Natl Acad Sci USA* 2003; 100: 8538-8542.

Ettlin DA, Zhang H, Lutz K, Jarmann T, Meier D, Gallo LM. Cortical activation resulting from painless vibrotactile dental stimulation measured by functional magnetic resonance imaging (fMRI). *J Dent Res* 2004; 83: 757-761.

Jantsch HHF, Kemppainen P, Ringler R, Handwerker HO, Forster C. Cortical representation of experimental tooth pain in humans. *Pain* 2005; 118: 390-399.

Azerad J, Woda A. Sensation evoked by bipolar intrapulpal stimulation in man. *Pain* 1977; 4: 145-152.

Kempainnen P, Pertovaara A, Huopaniemi T, Johansson G, Karonen SL. Modification of dental pain and cutaneous thermal sensitivity by physical exercise in man. *Brain Res* 1985; 360: 33–40.

Popovic MR. Transcutaneous electrical stimulation technology for functional electrical therapy applications. *Conf Proc IEEE Eng Med Biol Soc* 2006; 1: 2142-2145.

Keller T, Popovic MR, Pappas IPI, Muller PY. Transcutaneous functional electrical stimulator "Compex Motion". *Artificial Organs* 2002; 26: 219-223.

Frank H, Althoen SC. "The coefficient of variation." §C.4.b in Statistics: Concepts and Applications Cambridge, Great Britain: Cambridge University Press, 1995; 58-59.

Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ. Psychophysiological and modulatory interactions in neuroimaging. *NeuroImage* 1997; 6: 218–229.

Wagner KJ, Willoch F, Kochs EF, Siessmeier T, Tölle TR, Schwaiger M. Dose-dependent regional cerebral blood flow changes during remifentanil infusion in humans - A positron emission tomography study. *Anesthesiology* 2001; 94: 732-739.

Willoch F, Gamringer U, Medele R, Steude U, Tölle TR. Analgesia by electrostimulation of the trigeminal ganglion in patients with trigeminopathic pain: a PET activation study. *Pain* 2003; 103: 119–130.

Macaluso E, Frith CD, Driver J. Modulation of human visual cortex by crossmodal spatial attention. *Science* 2000; 289: 1206-1208.

Calvert GA. Crossmodal processing in the human brain: Insights from functional neuroimaging studies. *Cereb Cortex* 2001; 11:1110-1123.

Tracey I, Ploghaus A, Gati JS, Clare S, Smith S, Menon RS. Imaging attentional modulation of pain in the periaqueductal gray in humans. *J Neurosci* 2002; 22: 2748-2752.

Bantick SJ, Wise RG, Ploghaus A, Clare S, Smith SM, Tracey I. Imaging how attention modulates pain in humans using functional MRI. *Brain* 2002; 125: 310-319.

Valet M, Sprenger T, Boecker H, Willoch F, Rummeny E, Conrad B. Distraction modulates connectivity of the cingulo-frontal cortex and the midbrain during pain--an fMRI analysis. *Pain* 2004; 109: 399-408.

Seminowicz DA, Mikulis DJ, Davis KD. Cognitive modulation of pain-related brain responses depends on behavioral strategy. *Pain* 2004; 112: 48-58.

Schoedel A, Zimmermann K, Handerker HO, Forster C. The influence of stimulus ratings on cortical BOLD effects during painful and non-painful stimulation. *Pain* 2008; 135: 131-141

Price DD, Milling LS, Kirsch I, Duff A, Montgomery GH, Nicholls SS. An analysis of factors that contribute to the magnitude of placebo analgesia in an experimental paradigm. *Pain* 1999; 83:147-156.

Buchel C, Bornhovd K, Quante M, Glauche V, Bromm B, Weiller C. Dissociable neural responses related to pain intensity, stimulus intensity, and stimulus awareness within the anterior cingulate cortex: A parametric single-trial laser functional magnetic resonance imaging study. *J Neurosci* 2002; 22: 970-976.

Rainville P, Duncan GH, Price DD, Carrier B, Bushnell MC. Pain affect encoded in human anterior cingulate but not somatosensory cortex. *Science* 1997; 277: 968–971.

Tölle TR, Kaufmann T, Siessmeier T, Lautenbacher S, Berthele A, Munz F. Region-specific encoding of sensory and affective components of pain in the human brain: a positron emission tomography correlation analysis. *Annals of Neurology* 1999; 45: 40–47.

Damasio AR. Descartes' error: Emotion, reason, and the human brain. New York, Putnam's Sons, 1994.

7.2 STUDY 2

Taking sides with pain

Lateralization aspects related to cerebral processing of dental pain

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Published in:

Frontiers of Human Neuroscience

ABSTRACT

The current fMRI study investigated cortical processing of electrically induced painful tooth stimulation of both maxillary canines and central incisors in 21 healthy, right handed volunteers. A constant current, 150% above tooth specific pain-perception thresholds was applied and corresponding online ratings of perceived pain intensity were recorded with a computerized visual analog scale during fMRI measurements. Lateralization of cortical activations was investigated by a region of interest analysis. A wide cortical network distributed over several areas, typically described as the pain or nociceptive matrix, was activated on a conservative significance level. Distinct lateralization patterns of analyzed structures allow functional classification of the dental pain processing system. Namely, certain parts are activated independent of the stimulation site, and hence are interpreted to reflect cognitive emotional aspects. Other parts represent somatotopic processing and therefore reflect discriminative perceptive analysis. Of particular interest is the observed amygdala activity depending on the stimulated tooth that might indicate a role in somatotopic encoding.

Keywords: toothache; fMRI; dominance, cerebral; amygdala; cerebral cortex, lateralization

INTRODUCTION

Brain structures consistently activated by noxious stimuli are: anterior cingulate cortex (ACC), insula, secondary somatosensory cortex (SII), lentiform nuclei, cerebellum, and thalamus. Less consistently, activation related to nociception has been reported for primary somatosensory cortex (SI), motor cortex (M1), premotor areas, and subcortical structures (Treede et al., 1999; Petrovic et al., 2000; Peyron et al., 2000; Bingel et al., 2002; Farrell et al., 2005). Functionally, these areas have been divided into a lateral and medial pain system and substantial evidence has emerged in support of this model (Albe-Fessard et al., 1985; Bushnell et al., 1999; Tracey and Mantyh, 2007), although alternative hypotheses have also been put forward (Apkarian et al., 2005; Craig, 2005; Mouraux and Iannetti, 2009).

Generally, the medial pain system composed of the insular cortex, anterior cingulate, and limbic structures is held responsible for processing emotional-affective and cognitive-

behavioral pain aspects (Kulkarni et al., 2005; Wiech et al., 2006). The lateral pain system is attributed to sensory-discriminative components of pain and includes the lateral spinothalamic tract, the ventral posterolateral nucleus of the thalamus, and SI (Kenshalo Jr. et al., 1988; Bushnell and Duncan, 1989; Bushnell et al., 1999). In line with these functional attributes, one would expect to find evidence from experimental pain studies showing contralateral activation in this lateral system in response to unilateral noxious stimuli. Surprisingly, human imaging studies cannot consistently confirm activation in the lateral system in response to unilateral noxious stimuli. SI for example is only activated in approximately 50–75% of all reports (Bushnell et al., 1999; Peyron et al., 2000; Apkarian et al., 2005; Farrell et al., 2005). Similarly, hard evidence is lacking for distinct contralateral hemispheric activation of other structures of the lateral pain system. One explanation may be that only few studies report on administering noxious stimuli to bilateral homologous body parts (Coghill et al., 1999, 2001; Bingel et al., 2002, 2003; Brooks et al., 2002; Youell et al., 2004; Symonds et al., 2006). The current study aimed at elucidating cortical spatial representation and hemispheric lateralization in response to dental nociception.

Ideally, lateralization aspects of pain were investigated by asynchronously applying bilateral noxious stimuli at graded distances to the body midline. This is readily realized by stimulation of multiple teeth as previously done (Ettlin et al., 2004, 2009). A possible interference by midline crossing of maxillary nerve endings is unlikely based on findings by Kemppainen et al. (2003). Jantsch et al. (2005) published the first brain fMRI investigation on tooth pain induced by electric stimulation. However, they stimulated one single tooth only as well as the ipsilateral dorsal hand. The results of their study suggest that brain processing of electrically evoked dental pain shows similarities as well dissimilarities compared to upper extremity mechanically induced pain.

Based on the model of a lateral and medial pain system, we hypothesized that within the cortical pain circuitry, certain brain areas be activated dependent on the stimulation side and others showing lateralized or bilateral hemispheric activity independent of the side of stimulus application.

MATERIAL AND METHODS

PARTICIPANTS

21 neurologically healthy subjects (8 female/13 male, age 20-44, all right handed (Annett 1970) with no dental pain experience during the preceding year participated in the experiment. Inclusion criteria required test teeth to be caries free, vital, and without attachment loss. Dental and periodontal pathologies were excluded by professional dental and radiographic examinations of maxillary teeth. Subjects received detailed information about the experimental procedure and provided written informed consent. The study was approved by the local ethics committee and was conducted according to the guidelines of the Declaration of Helsinki for treatment of experimental human subjects.

EXPERIMENTAL MATERIAL

Maxillary alginate impressions were taken from the subjects' dentitions for fabrication of soft dental acrylic splints. Four pairs of stainless steel electrodes were embedded in each individual dental splint opposite the labial and palatal surface center of the target teeth, namely maxillary canines and central incisors (Fig. 1). They served as anode and cathode during electric stimulation. In order to minimize electric resistance during stimulation, a round piece of hydrogel (AMGEL Technologies, AG602-6, 8520 Lystrup, Denmark) with 3 mm diameter was placed between the tooth and anode and cathode, respectively, and was covered with a thin layer of toothpaste (Signal Micrograniuli, Unilever, Zug, Switzerland).

Electrical stimulation was performed by means of the portable system Compex Motion System (Keller et al., 2002) and the experimental protocol was controlled by the Presentation software (www.neurobs.com/presentation) via parallel port using a self made interface. To avoid radiofrequency contamination of the stimulation current, specially shielded wires were used. For rating of the stimulus intensities within the MRI scanner, a computerized visual analog scale was used (COVAS; MEDOC, Haifa, Israel), with anchor points "no pain" on the left and "worst imaginable pain" on the right. This COVAS was projected onto a screen outside the scanner, and a mirror based deflection system enabled its visibility for the subjects.

Figure 1: Customized acrylic splint with carbon wires and stainless steel electrodes (fabricated for each subject). Electrodes were placed on the labial and oral face of the respective tooth.

SENSORY TESTING PRIOR TO THE MR EXPERIMENT

One to two weeks prior to the MR experiment, sensory testing with the tooth stimulation setup was performed in order to assess individual thresholds for sensory perception (SPT), pain perception (PPT) and pain tolerance (PTT) separately for each target tooth. The three thresholds were defined as the average ascending electric stimulus intensity out of three tests at which the subject reported sensation, pain and pain tolerance, respectively. We also questioned subjects whether single stimuli were felt distinctly in one test tooth only, which was acknowledged by all participants. Sequence of tooth stimulation was randomized between individuals.

For all tooth stimuli (threshold determination and fMRI stimulation protocol) biphasic pulse forms of 1ms duration were applied on both maxillary canine and medial incisors with interstimulus intervals randomized between 7.5 to 10 seconds.

FMRI DATA ACQUISITION AND STIMULATION PROTOCOL

Within one to two weeks after sensory testing, subjects underwent the fMRI protocol in a Philips 3-T Achieva system (Philips Medical Systems, Best, The Netherlands) at the same time of day as threshold determination was performed, since evidence indicates a diurnal association of somesthesic perception (Fillingim and Ness, 2000; Sessle, 2000; Wiesenfeld-Hallin, 2005). Subjects were placed in the scanner in a supine position and their individual SPT and PPT were re-tested inside the scanner to exclude changes related to the experimental setting. No significant differences were observed (anova, greenhouse geisser corrected, $F = 1.653$, $p = 0.187$, $eta^2 = 0.076$. The fMRI stimulation protocol consisted of 40 constant stimuli per tooth applied in randomized order to the four teeth with an intensity 150% of the tooth specific PPT. Pain intensity ratings were used to control for differences in perceived pain intensity among tested teeth. For each tooth subjects were requested to rate the pain intensity of 10 randomly selected stimuli (25% of all stimuli applied). For those stimuli to be rated, the VAS appeared directly after stimulus delivery, and subjects were offered 5 seconds for pain intensity rating. For the remaining 75% of trials, the stimulus was followed by a fixation cross on the screen. We decided not to have every stimulus rated in order to minimize motion artifacts and other rating influences (Schoedel et al., 2008). All scans followed by a rating were therefore excluded from further fMRI analysis. The experimental run lasted approximately 23 minutes.

For the functional scans, a blood oxygen level dependent (BOLD) sensitive single-shot gradient echo planar imaging sequence was used with 33 axial slices, covering the entire cerebrum and cerebellum, using an 8 channel receive-only head coil. Parameters: echo time = 30 ms, flip angle = 75 degrees, repetition time = 2500 ms, slice thickness = 4 mm, inter-slice gap = 0 mm, field of view = 230 mm and matrix size in plane = 128 x 128, resulting in a voxel size of 1.72 x 1.72 x 4 mm^3. Three "dummy" scans were first acquired to reach steady state magnetization and discarded. 180 high-resolution T1 weighted axial slices (spoiled gradient echo) were acquired with TR = 20ms, flip angle = 20°, voxel size = 0.98 x 0.98 x 1.02 mm^3, FOV = 24 cm, matrix = 256 x 192, which were used as an underlay for individual functional maps.

DATA ANALYSIS

Individual pain perception thresholds were analyzed with respect to differences between the laboratory and fMRI condition in a repeated measures analysis of variance (RM-ANOVA), with the factors "location" and "tooth". A separate ANOVA with mean COVAS ratings per tooth as dependent variable, "tooth" as within-subject factor and “gender” as between-subject factor was calculated to check whether within each subject pain intensity and PPT varies between the stimulated teeth.

Functional image analysis was done using the SPM5 (http://www.fil.ion.ucl.ac.uk/spm) software package running on MatLab R2007a (Mathworks, Natiek, USA). In a first step, spatial realignment and reslicing to the first image in the series as reference was performed (detected movement did not exceed 1.5 mm (translational) or 1° (rotational) compared with the reference image). For studying group effects, data were normalized to the MNI template brain (Evans et al., 1993) followed by smoothing with a Gaussian kernel of 6 mm (FWHM) and scaled to the global mean intensity. A General Linear Model (GLM) was set up and estimated. Differences between stimulation and baseline were transformed into colour-coded T-maps for each voxel and superimposed onto the MNI single-subject-T1 brain. Corrected data (FWE) (Worsley et al., 1996) with $p < 0.01$ are reported in the general cortical activation section. Regions of interest (ROI) were defined, based on images provided by the "WFU-Pickatlas" (Lancaster et al., 1997 and 2000; Maldjian et al., 2003) for areas selected from pain literature reviews (Peyron et al., 2000; Farrell et al., 2005): postcentral gyrus, thalamus, amydgala, supramarginal (BA40), preparietal (BA5) and superior parietal (BA 7) areas, subcentral area (BA 43), cerebellum (anterior and posterior lobe), the supplemental motor area (BA6), frontomedial area (BA 46) and frontopolar (BA 10) areas, hippocampus, parahippocampus, caudate, putamen, pallidum and the brainstem. Two exceptions were applied: the "insula-ROI" provided by the WFU-Pickatlas was divided into three parts (anterior, medial and posterior) according to Brooks et al. (2002), since several reports suggest a complex anatomical (Varnavas and Grand 1999) and functional (Coghill et al.,1999; Brooks et al., 2002 and 2005; Symonds et al., 2006) fragmentation within the insula. To take into account the functional complexity of the cingulate cortex, we subdivided this structures based on Vogt, 2005. The numbers of activated voxels, mean- and maximum activation were calculated within each ROI.

Data were then analyzed using SPSS for Windows (SPSS Inc. Chicago, Illinois, Release 14.0.0). A reapeated measurement ANOVA (RM ANOVA) with “hemisphere” and “side of stimulation” as within-subjects-factors was performed for the ROIs. Main effects for factor "hemisphere" as well as interaction between factors “hemisphere” and “side of stimulation” were analyzed. For RM ANOVAS, results were Greenhouse-Geisser corrected for non-sphericity if applicable.

RESULTS

PSYCHOPHYSICS

Mean stimulus intensities of the general study population during the scanning procedure demonstrated a significant within-subjects effect (F = 3.45, p = 0.02) ranged from 20.76 to 25.24 mA across the four teeth, whereas respective ratings ranged from 46.9 to 49.1but showed no significant differences (F = 0.48, p = 0.70). According to gender related differences, we found a trend in the interaction gender * stimulus intensities (F = 2.74, eta^2 = 0.13, p = 0.051) but no interaction according to the gender * rating interaction with F = 0.87, eta^2 = 0.04 and p = 0.46 (for detailed information please see Table 1).

Table 1: Mean stimulus intensities and related mean ratings during fMRI in the overall study population and differentiated by gender.

Overall (n=21)	right		left	
	canine	central incisor	canine	central incisor
Stimulus intensities [mA]	25.2 ± 10.3	20.8 ± 11.3	24.8 ± 11.5	23.9 ± 13.1
COVAS ratings [0-100]	46.7 ± 18.5	48.0 ± 19.7	45.5 ± 19.0	46.9 ± 18.3
Female (n=8)				
Stimulus intensities [mA]	21.9 ± 9.6	17.0 ± 7.5	18.8 ± 7.5	15.8 ± 5.8
COVAS ratings [0-100]	38.0 ± 13.3	43.5 ± 20.2	39.5 ± 18.8	41.5 ± 17.6
Male (n=13)				
Stimulus intensities [mA]	27.3 ± 10.6	23.1 ± 12.8	28.5 ± 12.1	28.9 ± 13.9
COVAS ratings [0-100]	52.1 ± 19.6	50.8 ± 19.6	49.1 ± 18.8	50.2 ± 18.6

In the overall study population, post-hoc t-test on stimulus intensity revealed a significant difference between right central incisor and right canine (t = 3.82, p = 0.001) as well as between right central incisor and left canine (t = 2.83, p = 0.01). An additional one-way ANOVA exploring possible gender differences showed a significant difference between the stimulus intensities of the left central incisor (F= 6.30, p=0.2) and a trend with respect to the left canine (F=4.17, p=0.55). All other comparisons reached no significant level. All values are listed with respective standard deviations.

HEMODYNAMIC RESPONSES ACROSS THE ENTIRE BRAIN AND WITHIN REGIONS OF INTEREST

Group activation brain maps (stimulation vs. base-line) are displayed in Fig. 2 and specified in supplemental table 1 (as we focus on the lateralization analyses, we disclaim from describing this patterns here more extensively). All ROIs investigated showed significant activation compared to baseline, namely postcentral gyrus, thalamus, preparietal (BA5) and superior parietal (BA 7) areas, supramarginal (BA40) and subcentral areas (BA 43), anterior, medial and posterior insula, amydgala, hippocampus, parahippocampus, both cerebellae (anterior and posterior lobe), caudate, putamen, pallidum, supplementary motor (BA6), frontomedial (BA 46) and frontopolar areas (BA10) the subdivisions of the cingulate gyrus (PCC, pMCC, aMCC, pACC, sACC, and the brainstem) (Table 2).

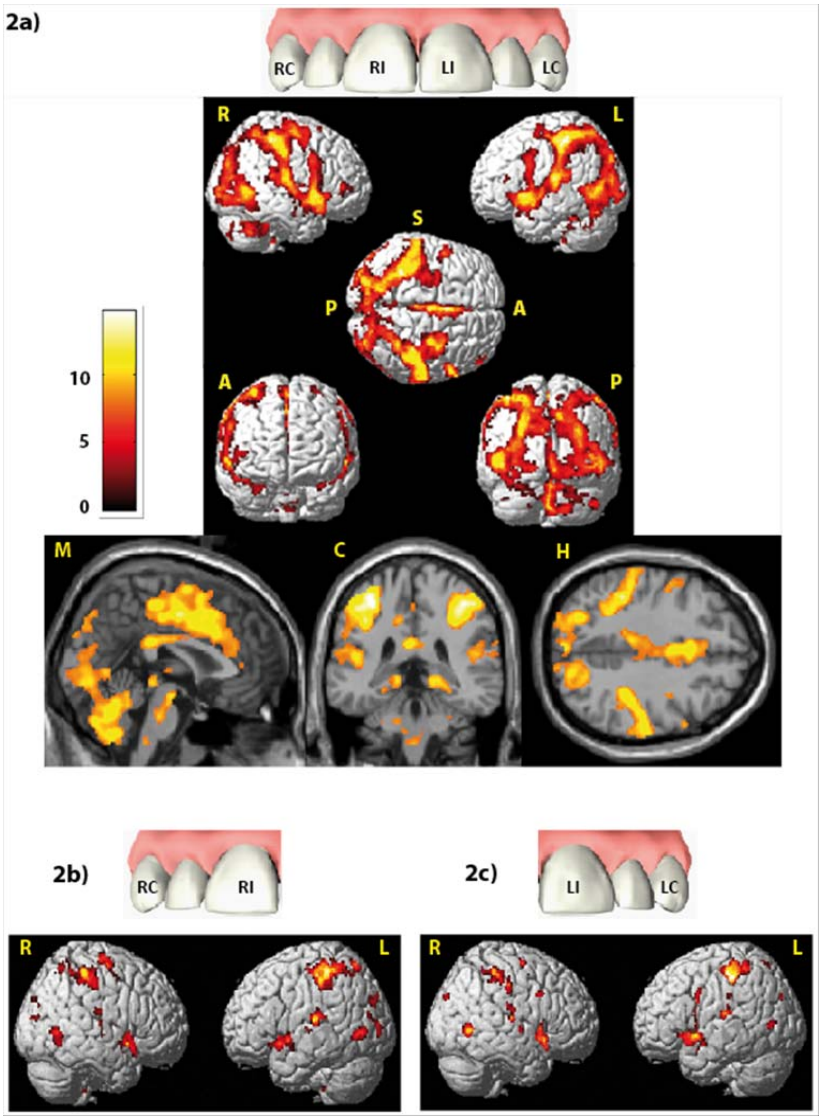

Figure 2:
Cortical areas activated by electrical tooth stimulation over all four teeth (2a) and with respect to both right teeth (RI and RC) and both left teeth (LI and LC) respectively (2b and 2c). Activity is projected onto the single-subject-MNI-template. Indicators at the rendered brains stand for the views: R=from right, L=from left, S=from superior, A=from anterior, P=from posterior, all brain figures are in neurological orientation. Slices from left to right: midsagital (M), coronal (C) at Y= -36 and horizontal (H) at Z= 54. Data are corrected for multiple comparison (FWE) p = 0.01 with an extended threshold of 10 voxel.

Table 2: Activation statistics in the selected regions of interest (see methods section) based on group analysis pooled across all four teeth. A small volume correction has been performed with the regions of interests as search volume. Described are cluster size, MNI coordinates of the maximally activated voxel with the respective p- and t-values. Data are family wise error (FWE) corrected ($p < 0.01$, extent voxel threshold $k = 10$).

Anatomical Description	Hemisphere	Cluster Size	MNI Coordinates (max T Voxel)	Voxel p (FWE-cor)	Voxel T (max T)
Postcentral Gyrus (SI)	L	970	-38 -36 54	0.000	14.74
	R	1024	50 -30 52	0.000	13.16
Thalamus	L	281	-10 -20 8	0.000	11.04
	R	275	14 -16 10	0.000	9.94
Preparietal Area (BA 5)	L	46	-34 -44 62	0.000	11.13
	R	11	32 -48 62	0.000	7.16
Superior Parietal Area (BA 7)	L	502	-22 -66 62	0.000	10.78
	R	387	16 -78 34	0.000	9.60
Supramarginal Area (BA 40)	L	586	-40 -36 58	0.000	13.66
	R	374	50 -30 50	0.000	12.16
Subcentral Area//SII (BA 43)	L	9	-52 -18 16	0.000	6.69
	R	36	66 -16 20	0.000	10.07
Anterior Insula	L	213	-46 12 -8	0.000	11.38
	R	215	42 16 -8	0.000	10.60
Medial Insula	L	490	-40 0 -10	0.000	11.13
	R	318	42 0 -10	0.000	9.85
Posterior Insula	L	52	-44 -14 2	0.000	7.93
	R	41	42 -12 -8	0.000	7.92
Amygdala	L	90	-20 0 -12	0.000	7.54
	R	72	26 2 -20	0.000	8.37
Hippocampus	L	79	-20 -24 -10	0.000	10.57
	R	48	18 -36 0	0.000	8.62
Parahippocampus	L	47	-24 -26 -16	0.000	7.80
	R	144	16 -38 -6	0.000	8.88
Cerebellum Anterior Lobe	L	617	-34 -58 -34	0.000	9.21
	R	1017	2 -62 -26	0.000	9.96
Cerebellum Posterior Lobe	L	617	-2 -72 -38	0.000	9.54
	R	1313	2 -64 -28	0.000	10.01
Caudate	L	117	-14 16 -8	0.000	10.36
	R	108	16 16 -10	0.000	8.88
Pallidum	L	69	-10 4 2	0.000	9.27
	R	6	16 10 -2	0.000	6.65
Putamen	L	455	-18 14 -2	0.000	14.55
	R	264	22 14 0	0.000	10.23
Supp_Motor_Area (BA 6)	L	631	-2 6 48	0.000	11.68
	R	421	2 8 46	0.000	10.71
Frontomedial Area (BA 46)	L	no suprathreshold cluster with this conservative statistic level			
	R	7	52 42 6	0.000	7.05
Frontopolar Area (BA 10)	L	no suprathreshold cluster with this conservative statistic level			
	R	1	52 42 0	0.000	6.41
PCC	L	280	-8 -28 44	0.000	10.70
	R	108	2 -28 52	0.000	9.02

pMCC	L	249	-2 -6 48	0.000	11.33
	R	259	8 -8 46	0.000	11.93
aMCC	L	521	-2 6 40	0.000	10.37
	R	480	2 16 38	0.000	9.83
pACC	L	103	-2 32 18	0.000	6.90
	R	20	2 34 20	0.000	6.75
sACC	L	no suprathreshold cluster with this conservative statistic level			
	R	no suprathreshold cluster with this conservative statistic level			
Brainstem	L	47	-2 -34 -50	0.000	6.83
	R	69	2 -26 -30	0.000	7.82

LATERALIZATION EFFECTS BASED ON REGIONS OF INTEREST ANALYSIS

1) There are no ROIs demonstrating a significant effect for “side of stimulation”.

2) There were several ROIs that were activated strongly on one hemisphere irrespective of the side of stimulation. Both, anterior and posterior cerebellar lobes demonstrated a stronger right hemispheric effect. A stronger left hemispheric effect was found in putamen, pregenual anterior cingulate cortex (pACC), supramarginal area (BA40) and parahippocampus (Table 3, Fig. 3).

3) There was one region, namely the subcentral area (BA 43), in which “hemisphere” showed a stronger right sided effect as well as an interaction with the factor “side of stimulation” (Table 3, Fig 3). This laterality effect was observed especially after left sided stimulation.

4) There were several regions in which no main effect but an interaction between “hemisphere” and “side of stimulation” was observed (Table 3, Fig 3). Postcentral gyrus (SI), posterior insula, thalamus and amygdala all showed a hemispheric dominance contralateral to the stimulation side.

Table 3: Repeated measures ANOVA results of the region of interest analysis. Only the significant and ($p<0.05$), trend-like interactions ($p < 0.10$) are shown (see Fig.3 for illustration). Main effect "tooth" is not shown, as there is neither a significant nor a trend within that factor. F = F-Value, p = p-value, eta^2 = proportion of the variability in the dependent measure that is attributable to a factor.

Anatomical Description	Main effect "hemisphere" F (eta^2) p	Interaction effect "tooth * hemisphere" F (eta^2) p
Thalamus	0.028 (0.001) 0.870	11.038 (0.356) **0.003**
Postcentral_Gyrus (SI)	0.876 (0.042) 0.360	12.928 (0.393) **0.002**
Posterior Insula	0.003 (0.000) 0.959	4.564 (0.186) **0.045**
Amygdala	3.615 (0.153) 0.072	23.163 (0.537) **0.000**
Subcentral Area (BA 43)	17.723 (0.470) **0.000**	12.899 (0.392) **0.002**
Preparietal Area (BA5)	1.219 (0.057) 0.283	3.008 (0.131) *0.098*
Cerebellum (posterior lobe)	18.814 (0.485) **0.000**	1.349 (0.063) 0.259
Cerebellum (anterior lobe)	4.546 (0.185) **0.046**	1.942 (0.089) 0.179
Parahippocampus	6.628 (0.249) **0.018**	1.417 (0.066) 0.248
Supramarginal Area (BA 40)	7.191 (0.264) **0.014**	1.654 (0.076) 0.213
Pregenual Anterior Cingulate (pACC)	13.934 (0.411) **0.000**	0.771 (0.037) 0.515
Anterior medial Cingulate (aMCC)	4.271 (0.176) *0.052*	0.507 (0.025) 0.679
Putamen	7.213 (0.265) **0.014**	0.718 (0.035) 0.407
Supplementary Motor Area (BA 6)	3.909 (0.163) *0.062*	0.357 (0.018) 0.557

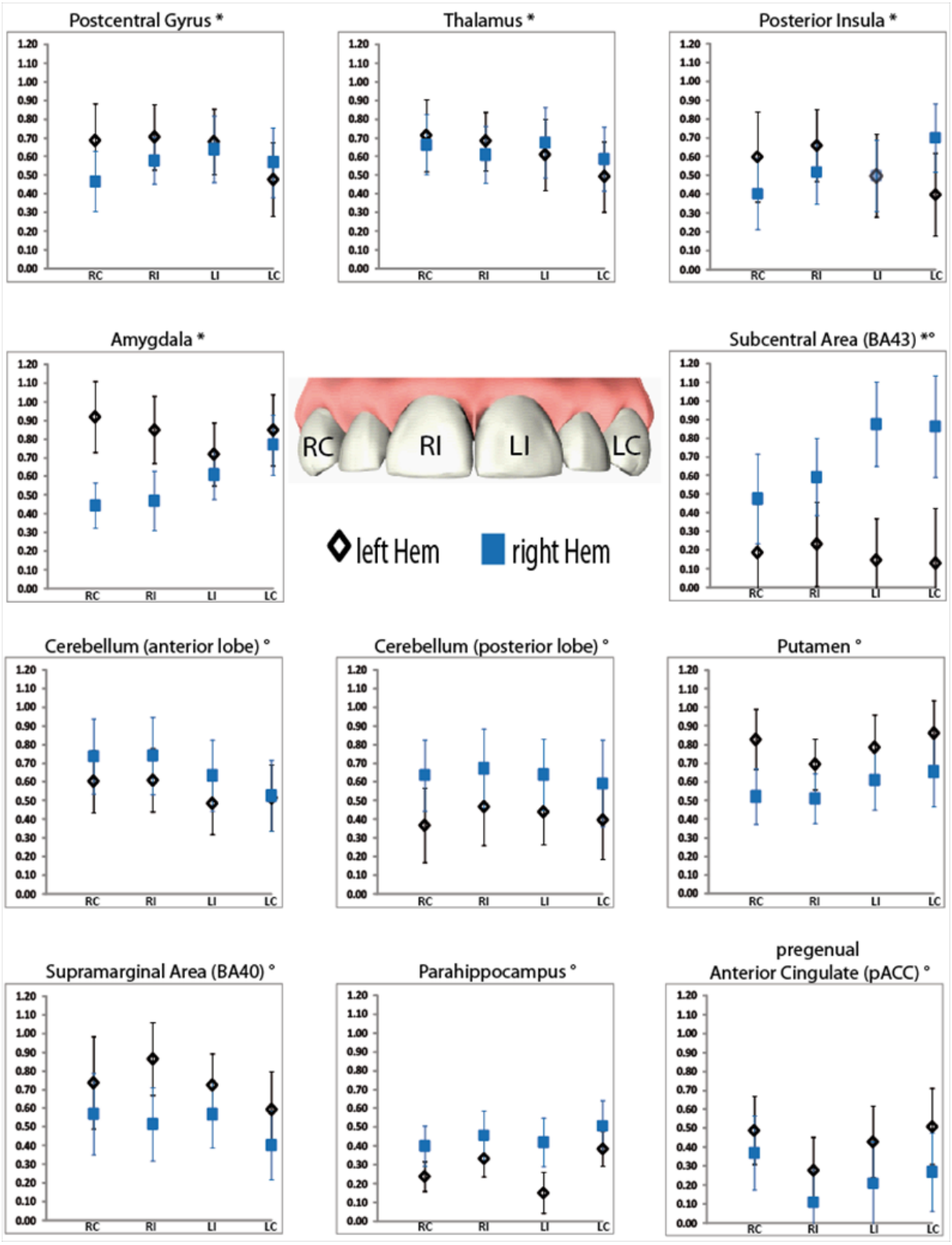

Figure 3:

Regions of interest (ROIs) showing significant main effect hemisphere (indexed with °) or interaction (indexed with *) in the repeated measure ANOVA. Displayed are mean activations (Y axis) with corresponded standard errors for each tooth within the respective hemisphere. RC = right canine, RI = right central incisor, LI = left central incisor, LC = left canine.

DISCUSSION

The aim of this study was to elucidate cortical spatial representation and hemispheric lateralization in response to noxious electric dental stimulation. Findings reveal robust brain activation in areas previously shown to be involved in pain processing.

Focusing on lateralization aspects, we categorize the findings into three groups 1) structures exhibiting hemispheric lateralization irrespective of side of stimulation 2) structures showing activation dominance contralateral to the side of stimulation without hemispheric lateralization 3) structures demonstrating not only hemispheric lateralization, but also dependency on side of stimulation. In the following, we discuss these findings in detail.

HEMISPHERIC LATERALIZATION IRRESPECTIVE OF SIDE OF STIMULATION

We found evidence for hemispheric lateralization in six brain areas irrespective of side of stimulation. The anterior and posterior cerebellar lobes as well as the parahippocampus demonstrate a stronger right hemispheric effect, whereas a stronger left hemispheric effect was observed in putamen, pregenual cingulate cortex, and supramarginal area (BA 40).

Pain related cerebellar activity has been consistently demonstrated (reviewed in Peyron et al., 2000; Apkarian et al., 2005; Farrell et al., 2005) and several suggestions are published in order to explain this often robust activity (see e.g., Saab and Willis, 2003). Evidence for direct and/or collateral trigeminal input to cerebellar structures is provided by animal studies (Snyder et al., 1978; Dietrichs and Walberg, 1987; Patrick and Robinson, 1987; Saab et al., 2001; Bukowska et al., 2006; Holtzman et al., 2006). Findings revealed that trigeminal brainstem nuclei interpolaris, oralis, and principalis project predominantly ipsilateral to cerebellar regions. Taken together, cerebellar cortices receive mostly ipsilateral and to a lower extend, bilateral fibers from several trigeminal brainstem nuclei (detailed summarized by Dietrichs and Walberg, 1987). Recent work by Borsook et al. (2008) provides an overview of 28 studies with cerebellar activation in acute experimental pain using fMRI and PET. Bilateral activity is described in 15, ipsilateral activity in 10, and contralateral activity in 3 of them. This is an astonishing observation as most of the reviewed investigations stimulated the upper extremities unilaterally. Considering the anatomical perspective provided by animal research, one would expect a predominantly ipsilateral and to a smaller extent, bilateral activation. They also summarize own research on investigating specifically noxious and non-noxious thermal heat and brush stimuli applied to the maxillary division of the face

in healthy and neuropathic pain patients. Summarized, noxious heat evoked predominantly contralateral activation in both groups, while brush evoked more ipsilateral cerebellar activity. Based on their observations a “dichotomy of innocuous stimuli/sensorimotor cerebellum activation versus noxious experience/cognitive/limbic cerebellum activation” was suggested.

Our data show a right-lateralized effect in both, anterior and posterior cerebellum as well as in the parahippocampus. Schmahmann and Pandya (1997) as well as Manto (2006) describe outputs to numerous (limbic) structures, among them; hippocampal complex, amygdala, thalamic nuclei, hypothalamus, and the periaqueductal gray. Based on these connections, the cerebellum has also been called “modulator of different neurologic functions,” thus directly influencing sensory, but also emotional and cognitive processing (Allen et al., 2005; Ito, 2008).

The role of the basal ganglia in processing nociceptive information is still debated despite their robustly observed involvement shown in human studies (Coghill et al., 1999, 2001; Apkarian et al., 2005) as well as in animal research (Chudler, 1998). Neuroanatomical evidence reveals afferents from several subdivisions of the cerebral cortex (including neocortical and cingulate cortex), thalamic nuclei, cerebellum, the amygdala, parabrachial area, and dorsal raphe nucleus (Chudler and Dong, 1995; Downar et al., 2003). Although the main role of the basal ganglia is often related to sensorimotor integration and thus adaptation of motor responses to noxious stimuli, their involvement in other dimensions of pain processing cannot be excluded. The review of Chudler and Dong (1995) provides strong evidence for a functional involvement of the basal ganglia in both, direct innocuous and noxious somatosensory processing. Supporting this finding, Coghill et al. (1999) pointed out the role of the putamen and globus pallidus (bilateral) in processing of human pain intensity and Scott et al. (2006) linked the role of the putamen to anticipatory mechanisms. Publications of several other investigations suggest cerebellar and basal ganglia processing to depend on cognitive functions (Akshoomoff and Courchesne, 1992; Schmahmann and Pandya, 1997; Schmahmann and Caplan, 2006). However, based on present literature no evidence emerges regarding lateralization of cognitive functions in these areas. Therefore, we do not assume that left-lateralization found in our data indicates cognitive involvement, but rather reveals motor functions, many of which are known to be lateralized to the motor dominant hemisphere. This interpretation is up for debate as two previous studies revealed

certain aspects of hemispheric dominance to be independent of handedness for noxious and non-noxious somatosensory stimulation (Jung et al., 2003; Schlereth et al., 2003).

Focusing on significantly activated cingulate cortex subdivisions (PCC, pMCC, aMCC, pACC, and sACC) we found a left hemispheric lateralization in the pACC and a trend toward left-lateralization in the aMCC, but no lateralization in the more posterior divisions. Current literature indicates that pACC is associated with engaging in positively valenced events and is linked with the amygdala's lateral basal and accessory basal nuclei, whereas the aMCC contains the rostral cingulate motor area (Vogt, 2005). Based on their findings, Büchel et al. (2002) concluded that a main function of the ACC's subdivisions is to integrate a wide range of pain relevant information and to generate adequate responses. However, considering pain related investigations, distinct lateralization aspects of ACC subdivisions have to date not been in the focus of interest. In line with its functional attributes (selection of adequate reactions), the aMCC activation pattern found in our study points toward involvement in motor components of nociception, as seen for cerebellum and putamen (Vogt, 2005).

The left-lateralization effect noticed in the supramarginal area (BA 40) may also relate to a functional role of this structure in sensorimotor integration (Serrien et al., 2006), or a specialization for the detection of behaviorally relevant stimuli (Corbetta and Shulman, 2002).

Even if the stimuli may not be interpreted by subjects as potentially dangerous, pain is inherently salient (Legrain et al., 2009). Conform to Farrer et al. (2008) we favor an interpretation that the left lateralized activation within the supramarginal area is related to the analysis and integration of body-related nociceptive sensations in contrast to right-lateralized parietal cortex activity which is thought to mediate the analysis and integration of body-related visual and painless somatosensory information.

With respect to the finding that parahippocampus shows predominantly right sided BOLD responses to dental nociceptive stimuli, the function of this structure may also be described in the context of novelty detection theories, as suggested before by Bingel et al. (2002) and Ploghaus et al. (2000) and corroborated by Strange and Dolan (2006) with fear related stimuli.

STRUCTURES WITH PREDOMINANT CONTRALATERAL ACTIVATION

We found evidence in five brain areas that reveal activation dominance contralateral to the side of stimulation: SI, thalamus, posterior insula, amygdala, and subcentral area (BA 43). Subcentral area additionally demonstrates hemispheric lateralization and will be discussed later.

Contralateral activation is closely linked to somatotopic encoding. Yet, unresolved questions exist as to lateralization aspects in cortical structures like SI, SII, thalamus, and posterior insula. To address this topic was one of the aims of the present study. Previously, Bingel et al. (2003) have investigated lateralized brain activity in response to noxious stimuli in SI, SII, insula, and thalamus and found contralateral bias in all these four areas. Although stimulation of either hand evoked bilateral activation of anterior and posterior insular regions, a contralaterally biased response was found for the posterior parts of the insula bordering SII. Similar findings were reported by Brooks et al. (2002) who applied noxious thermal stimuli to both hands. Again, activation in insular posterior parts was dependent on the site of stimulation, whereas this dependency was absent in more anterior insular areas and SII. Interestingly, activation was absent in thalamus and SI. If activation in the thalamus is reported, then mostly contralateral but also often bilateral (Peyron et al., 2000) although, more recently, Kulkarni et al. (2005) reported ipsilateral, but no contralateral thalamus activity.

Our electric dental stimulation data show robustly that SI is activated bilaterally with a significant predominance contralateral to the stimulus application side. The same findings hold true for thalamus, and posterior insular cortex (Figure 3). We thus confirm the functional role of these cortical areas in topographic stimulus encoding.

Possibly, lateralized activation of areas could be caused by evasive or protective motor action dependent on the site of stimulation. However, this unlikely explains the present data, since withdrawal and orientation responses have been shown to predominantly activate cingulate cortex subdivisions (Vogt, 2005; Peyron et al., 2007) and cerebellum (Dimitrova et al., 2003) but not SI, thalamus, posterior insula, amygdala, or subcentral area (BA 43).

The amygdala's involvement in various forms of conditioned hypoalgesia and analgesia has been well established in several animal studies (e.g., Crown et al., 2000; Neugebauer and Li, 2002, 2003; Neugebauer et al., 2004). Lesion studies, specifically of the latero-capsular

amygdaloid nucleus (also termed “nociceptive amygdala”) demonstrated reduced or completely abolished conditioned behavior (Watkins et al., 1998). Inconsistent amygdala activation in response to nociceptive and other aversive stimuli in humans is frequently reported (Baas et al., 2004; Phan et al., 2004; Rempel-Clower, 2007; Tracey and Mantyh, 2007). Why amygdala activation appears robustly in response to noxious dental stimulation in comparison to stimulation of other body parts (Peyron et al., 2000; Apkarian et al., 2005; Farrell et al., 2005) can only be speculated. One possible explanation is that the amygdala has proven relevant for emotional conditioning (Büchel et al., 1999; Büchel and Dolan, 2000; Cardinal et al., 2002) and thus, a unique emotional salience of dental pain could explain our findings. However, it must be noted that the emotional value of the applied stimuli has not been directly controlled for. Stimulus conditioning and (missing) previous dental pain experiences could both contribute to an assumed peculiarity of dental pain. Alternatively dental pain may involve different processing pathways (trigeminal versus spinal). Future investigations need to further elucidate this topic.

Lateralization of amygdala activation shows an inconsistent picture. Among human neuroimaging studies, none described a clearly lateralized activation dependent on the stimulation side (e.g., Bingel et al., 2002; Bornhovd et al., 2002). The present data show that BOLD signal in the amygdala is stronger contralateral than ipsilateral to the side of stimulation. To the best of our knowledge, this has previously not been shown in pain studies nor in investigations on emotion. Regarding the latter, Baas et al. (2004) pointed out that there is no stimulation side dependent amygdala lateralization effect across 54 studies analyzed by them. One has of course to consider different paradigms and also different statistical approaches which hamper an adequate conclusion so far. Our approach of analyzing mean activations by a RM-ANOVA provides some evidence toward possible somatotopic related encoding properties. Previous studies may have missed a lateralization effect in the amygdala due to less salient stimuli and/or bigger voxel sizes (introducing greater partial volume effects and hence reduced statistical power).

Interestingly, contrary to previous reports our data do not indicate lateralization of brainstem activity. We propose that this is due to methodological reasons. Without applying special imaging techniques, brainstem activity is often severely masked by movement artifacts stemming from pulsation movements of the A. carotis. Correction of these artifacts involves, e.g., cardiac triggering, which we did not apply for sake of greater power in the

remaining regions. Methods to deal with physiological artifacts *post hoc* (see e.g., Harvey et al., 2008) were also not applicable due to missing cardiac and respiratory information. Thus we argue that brainstem effects are likely to be missed in our study which should not give rise to suspicion regarding the effects found.

STRUCTURES SHOWING HEMISPHERIC DOMINANCE AND PREDOMINANT CONTRALATERAL ACTIVATION

The subcentral area (BA 43) shows significant lateralization to one hemisphere (main effect "hemisphere") and also significant enhanced activation contralateral to the stimulus. Interestingly, this area is not frequently reported in pain studies. Subcentral area (BA 43) is located at the ventral end of the pre/postcentral gyri and the bank of the lateral sulcus and also delineated as SII. Its rostral and caudal borders are neighbored by both, the anterior and posterior subcentral sulci. Its distinction from surrounding areas is based on its specific cytoarchitectonic features already observed by Brodman (Eickhoff et al., 2006 and 2007).

Only few human studies explicitly reported lateralized activation within BA 43 in response to noxious stimulation. Becerra et al. (2001) noted right-lateralized activation in BA 43 in response to noxious thermal hand stimulation, but this result was not addressed in the discussion. Focussing on idiopathic chronic low back pain, Giesecke et al. (2004) found bilateral activation in BA 43 and discussed it as being part of the secondary somatosensory cortex. In a simultaneous EEG-fMRI investigation, Christmann et al. (2007) reported bilateral activation within BA 43 and also delineated it as being part of SII. However, in none of these studies, activity within BA 43 was further interpreted by the authors.

The present data showed a strong hemodynamic response within BA 43, with a significant interaction effect between stimulated tooth and hemisphere (activity is predominantly contralateral to the stimulus) as well as a main effect towards the right hemisphere (Table 3 and Fig. 3). This distinct right-lateralized activation is eye-catching and the present data may shed new light on the role of this structure, since the activation pattern is quite different from other parts of SII. Strong anatomical connections between the subcentral area and pre-motor cortices, as well as posterior parietal area (Cipolloni and Pandya, 1999) place the subcentral area (BA 43) in an ideal position for multimodal sensorimotor integration. Such a

role has long been suggested for mammals (Krubitzer, 1996) and more recently for humans (Disbrow et al., 2000).

Although our results point towards a specialized somatosensory encoding function with a possible role in sensorimotor integration, it may be premature to speculate on the specific role of BA 43 within the pain circuitry.

STUDY LIMITATIONS

A full understanding of brain activations in response to painful stimuli is inherently limited by the complexity of the multidimensional pain experience. Some brain activity patterns may not necessarily be directly involved in pain processing, but rather relate to aspects of alertness and/or orientation responses. Namely parieto-occipital activation clusters may be interpreted in this way. The human pain experience implies orientation toward pain and toward options to relieve it. Some brain activity may thus not be directly linked to the pain experience itself. Furthermore, as the intensities of all stimuli were above the pain threshold, purely somatosensory processes cannot be controlled for and thus it cannot be excluded that some brain activities may reflect somatosensory aspects of the stimulation. Finally, although all subjects located their pain to the stimulation tooth, we are unable to report on the fiber subpopulations involved in pain transmission.

CONCLUSIONS

Electrically evoked dental pain activates cortical areas typically described in spinal pain studies. Yet, robust activation can be observed in additional areas, namely the amygdala. Besides previously known lateralization effects, hemispheric lateralization irrespective of side of stimulation were observed in subdivisions of the ACC (aMCC and pACC). Predominant contralateral activation in the posterior insular cortex and the amygdala points towards their possible involvement in somatotopic encoding of noxious stimuli, in addition to other, previously described functions.

CONFLICT OF INTEREST STATEMENT

All authors declare that this study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

ACKNOWLEDGMENTS

This work was supported by GlaxoSmithKline, Division of consumer health care, the Swiss Dental Association and the Swiss Society for Endodontology.

SUPPLEMENTAL MATERIAL

Supplementary table 1: Complete list of local maxima within clusters activated for the contrast stimulation versus baseline (as illustrated in Fig. 2). As there are very large clusters, anatomical descriptions are related only to the maximally activated voxel within each cluster.

Anatomical Description	Cluster Size	Voxel p (FWE-cor)	MNI Coordinates (max T Voxel)	Voxel T (max T)
Postcentral Gyrus	27749	0.000	14.74	-38 -36 54
		0.000	14.55	-18 14 -2
		0.000	14.21	-40 -28 56
		0.000	13.92	-38 -32 62
		0.000	13.16	50 -30 52
		0.000	13.13	38 -34 48
		0.000	12.85	44 -62 4
		0.000	12.65	-34 -44 56
		0.000	12.52	-34 -42 52
		0.000	12.5	42 -36 54
		0.000	12.43	-42 -32 46
		0.000	12.34	-46 12 -10
		0.000	12.25	-46 -20 58
		0.000	12.05	-56 -28 52
		0.000	11.75	-42 -28 18
		0.000	11.7	-58 -22 14
		0.000	11.56	58 12 -8
		0.000	11.55	-52 -22 54
		0.000	11.55	40 2 -18
		0.000	11.46	-54 -26 16
		0.000	11.44	-40 -36 42
		0.000	11.43	54 -22 44
		0.000	11.43	34 -8 64
		0.000	11.38	36 -18 66
		0.000	11.18	-40 0 -12
		0.000	11.15	50 16 -14
		0.000	11.04	-10 -20 8
		0.000	11.02	-52 4 -6

		0.000	10.98	-56 6 -2
		0.000	10.85	-46 4 -4
		0.000	10.84	36 -46 54
		0.000	10.78	-22 -66 62
Posterior Cingulate	3304	0.000	13.16	-4 -32 26
		0.000	11.68	0 6 48
		0.000	11.35	-2 -14 56
		0.000	11.29	-2 -2 48
		0.000	11.09	-2 16 38
		0.000	10.82	2 -8 46
		0.000	10.24	-8 -28 44
		0.000	9.98	0 -26 54
		0.000	9.96	-2 -6 56
		0.000	9.95	-2 18 46
		0.000	9.11	2 -22 44
		0.000	8.96	2 -2 66
		0.000	8.87	4 -2 38
		0.000	8.82	10 22 28
		0.000	8.75	-10 18 30
		0.000	8.2	-2 30 18
		0.000	7.74	6 -4 30
		0.000	7.58	8 16 64
		0.000	7.31	4 -20 28
		0.000	6.92	-4 26 38
		0.000	6.86	2 -14 28
		0.000	6.48	-2 38 10
		0.003	6.02	10 4 40
Midbrain	99	0.000	10.17	2 -16 -14
		0.000	7.4	4 -26 -28
		0.000	7.2	0 -18 -20
Cerebellum posterior lobe	117	0.000	9.6	14 -76 -48
Medulla	72	0.000	8.07	-2 -34 -50
		0.000	6.48	-6 -34 -42
Inferior Frontal Gyrus	37	0.000	7.53	52 44 4
		0.001	6.25	54 40 -2
Cerebellum posterior lobe	26	0.000	7.19	32 -80 -32
Temporal inferior Lobe	31	0.000	6.89	-44 -42 -28
		0.000	6.51	-38 -48 -24
		0.002	6.11	-48 -42 -26
Cingulate Gyrus	13	0.000	6.79	14 -28 36
Occipital Lobe (Lingual)	55	0.000	6.69	2 -68 4
		0.000	6.53	6 -64 0
Parietal Lobe (Precuneus)	17	0.000	6.62	-6 -52 52
		0.001	6.25	-2 -58 52
Inferior Parietal Lobe (Supramarginal)	11	0.001	6.2	68 -36 26

REFERENCES

Akshoomoff, N. A., and Courchesne, E. (1992). A new role for the cerebellum in cognitive operations. *Behav. Neurosci.* 106, 731–738.

Albe-Fessard, D., Berkley, K. J., Kruger, L., Ralston, H. J. III, and Willis, W. D. Jr. (1985). Diencephalic mechanisms of pain sensation. *Brain Res.* 356, 217–296.

Allen, G., McColl, R., Barnard, H., Ringe, W. K., Fleckenstein, J., and Cullum, C. M. (2005). Magnetic resonance imaging of cerebellar–prefrontal and cerebellar–parietal functional connectivity. *Neuroimage* 28, 39–48.

Annett, M. (1970). A classification of hand preference by association analysis. *Br. J. Psychol.* 61, 303–321.

Apkarian, A. V., Bushnell, M. C., Treede, R. D., and Zubieta, J. K. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *Eur. J. Pain* 9, 463–484.

Baas, D., Aleman, A., and Kahn, R. S. (2004). Lateralization of amygdala activation: a systematic review of functional neuroimaging studies. *Brain Res. Rev.* 45, 96–103.

Becerra, L., Breiter, H. C., Wise, R., Gonzalez, R. G., and Borsook, D. (2001). Reward circuitry activation by noxious thermal stimuli. *Neuron* 32, 927–946.

Bingel, U., Quante, M., Knab, R., Bromm, B., Weiller, C., and Büchel, C. (2002). Subcortical structures involved in pain processing: evidence from single-trial fMRI. *Pain* 99, 313–321.

Bingel, U., Quante, M., Knab, R., Bromm, B., Weiller, C., and Büchel, C. (2003). Single trial fMRI reveals significant contralateral bias in responses to laser pain within thalamus and somatosensory cortices. *Neuroimage* 18, 740–748.

Bornhovd, K., Quante, M., Glauche, V., Bromm, B., Weiller, C., and Büchel, C. (2002). Painful stimuli evoke different stimulus–response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. *Brain* 125(Pt 6), 1326–1336.

Borsook, D., Moulton, E. A., Tully, S., Schmahmann, J. D., and Becerra, L. (2008). Human cerebellar responses to brush and heat stimuli in healthy and neuropathic pain subjects. *Cerebellum* 7, 252–272.

Brooks, J. C., Nurmikko, T. J., Bimson, W. E., Singh, K. D., and Roberts, N. (2002). fMRI of thermal pain: effects of stimulus laterality and attention. *Neuroimage* 15, 293–301.

Brooks, J. C., Zambreanu, L., Godinez, A., Craig, A. D., and Tracey, I. (2005). Somatotopic organisation of the human insula to painful heat studied with high resolution functional imaging. *Neuroimage* 27, 201–209.

Büchel, C., Bornhovd, K., Quante, M., Glauche, V., Bromm, B., and Weiller, C. (2002). Dissociable neural responses related to pain intensity, stimulus intensity, and stimulus awareness within the anterior cingulate cortex: a parametric single-trial laser functional magnetic resonance imaging study. *J. Neurosci.* 22, 970–976.

Büchel, C., and Dolan, R. J. (2000). Classical fear conditioning in functional neuroimaging. *Curr. Opin. Neurobiol.* 10, 219–223.

Büchel, C., Dolan, R. J., Armony, J. L., and Friston, K. J. (1999). Amygdala–hippocampal involvement in human aversive trace conditioning revealed through event-related functional magnetic resonance imaging. *J. Neurosci.* 19, 10869–10876.

Bukowska, D., Mierzejewska-Krzyzowska, B., and Zguczyński, L. (2006). Topography and axonal collaterals of trigeminocerebellar projection to the paramedian lobule and uvula in the rabbit cerebellum. *Acta Neurobiol. Exp. (Wars)* 66, 145–151.

Bushnell, M. C., and Duncan, G. H. (1989). Sensory and affective aspects of pain perception: is medial thalamus restricted to emotional issues? *Exp. Brain Res.* 78, 415–418.

Bushnell, M. C., Duncan, G. H., Hofbauer, R. K., Ha, B., Chen, J. I., and Carrier, B. (1999). Pain perception: is there a role for primary somatosensory cortex? *Proc. Natl. Acad. Sci. U.S.A.* 96, 7705–7709.

Cardinal, R. N., Parkinson, J. A., Lachenal, G., Halkerston, K. M., Rudarakanchana, N., Hall, J., Morrison, C. H., Howes, S. R., Robbins, T. W., and Everitt, B. J. (2002). Effects of selective excitotoxic lesions of the nucleus accumbens core, anterior cingulate cortex, and central nucleus of the amygdala on autoshaping performance in rats. *Behav. Neurosci.* 116, 553–567.

Christmann, C., Koeppe, C., Braus, D. F., Ruf, M., and Flor, H. (2007). A simultaneous EEG-fMRI study of painful electric stimulation. *Neuroimage* 34, 1428–1437.

Chudler, E. H. (1998). Response properties of neurons in the caudate-putamen and globus pallidus to noxious and non-noxious thermal stimulation in anesthetized rats. *Brain Res.* 812, 283–288.

Chudler, E. H., and Dong, W. K. (1995). The role of the basal ganglia in nociception and pain. *Pain* 60, 3–38.

Cipolloni, P. B., and Pandya, D. N. (1999). Cortical connections of the frontoparietal opercular areas in the rhesus monkey. *J. Comp. Neurol.* 403, 431–458.

Coghill, R. C., Gilron, I., and Iadarola, M. J. (2001). Hemispheric lateralization of somatosensory processing. *J. Neurophysiol.* 85, 2602–2612.

Coghill, R. C., Sang, C. N., Maisog, J. M., and Iadarola, M. J. (1999). Pain intensity processing within the human brain: a bilateral, distributed mechanism. *J. Neurophysiol.* 82, 1934–1943.

Corbetta, M., and Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nat. Rev. Neurosci*. 3, 201–202.

Craig, A. D. (2005). Forebrain emotional asymmetry: a neuroanatomical basis? *Trends Cogn. Sci.* 9, 566–571.

Crown, E. D., King, T. E., Meagher, M. W., and Grau, J. W. (2000). Shock-induced hyperalgesia: III. Role of the bed nucleus of the stria terminalis and amygdaloid nuclei. *Behav. Neurosci.* 114, 561–573.

Dietrichs, E., and Walberg, F. (1987). Cerebellar nuclear afferents – where do they originate? A re-evaluation of the projections from some lower brain stem nuclei. *Anat. Embryol.* 177, 165–172.

Dimitrova, A., Kolb, F. P., Elles, H.-G., Maschke, M., Forsting, M., Diener, H. C., and Timmann, D. (2003). Cerebellar responses evoked by nociceptive leg withdrawal reflex as revealed by event-related fMRI. *J. Neurophysiol.* 90, 1877–1886.

Disbrow, E., Roberts, T., and Krubitzer, L. (2000). Somatotopic organization of cortical fields in the lateral sulcus of *Homo sapiens*: evidence for SII and PV. *J. Comp. Neurol.* 418, 1–21.

Downar, J., Mikulis, D. J., and Davis, K. D. (2003). Neural correlates of the prolonged salience of painful stimulation. *Neuroimage* 20, 1540–1551.

Eickhoff, S. B., Amunts, K., Mohlberg, H., and Zilles, K. (2006). The human parietal operculum. II. Stereotaxic maps and correlation with functional imaging results. *Cereb. Cortex* 16, 268–279.

Eickhoff, S. B., Grefkes, C., Zilles, K., and Fink, G. R. (2007). The somatotopic organization of cytoarchitectonic areas on the human parietal operculum. *Cereb. Cortex* 17, 1800–1811.

Ettlin, D. A., Brügger, M., Keller, T., Luechinger, R., Jäncke, L., Palla, S., Barlow, A., Gallo, L. M., and Lutz, K. (2009). Interindividual differences in the perception of dental stimulation and related brain activity. *Eur. J. Oral Sci.* 1, 27–33.

Ettlin, D. A., Zhang, H., Lutz, K., Jarmann, T., Meier, D., Gallo, L. M., Jancke, L., and Palla, S. (2004). Cortical activation resulting from painless vibrotactile dental stimulation measured by functional magnetic resonance imaging (fMRI). *J. Dent. Res.* 83, 757–761.

Evans, A. J., Iwai, F., Grist, T. A., Sostman, H. D., Hedlund, L. W., Spritzer, C. E., Negro-Vilar, R., Beam, C. A., and Pelc, N. J. (1993). Magnetic resonance imaging of blood flow with a phase subtraction technique: in vitro and in vivo validation. *Invest. Radiol.* 28, 109–115.

Farrell, M. J., Laird, A. R., and Egan, G. F. (2005). Brain activity associated with painfully hot stimuli applied to the upper limb: a meta-analysis. *Hum. Brain Mapp.* 25, 129–139.

Farrer, C., Frey, S. H., van Horn, J. D., Tunik, E., Turk, D., Inati, S., and Grafton, S. T. (2008). The angular gyrus computes action awareness representations. *Cereb. Cortex* 18, 254–261.

Fillingim, R. B., and Ness, T. J. (2000). Sex-related hormonal influences on pain and analgesic responses. *Neurosci. Biobehav. Rev.* 24, 485–501.

Giesecke, T., Gracely, R. H., Grant, M. A., Nachemson, A., Petzke, F., Williams, D. A., and Clauw, D. J. (2004). Evidence of augmented central pain processing in idiopathic chronic low back pain. *Arthritis Rheum.* 50, 613–623.

Harvey, A. K., Pattinson, K. T. S., Brooks, J. C. W., Mayhew, S. D., Jenkinson, M., and Wise, R. G. (2008). Brainstem functional magnetic resonance imaging: disentangling signal from physiological noise. *J. Magn. Reson. Imaging* 28, 1337–1344.

Holtzman, T., Rajapaksa, T., Mostofi, A., and Edgley, S. A. (2006). Different responses of rat cerebellar Purkinje cells and Golgi cells evoked by widespread convergent sensory inputs. *J. Physiol. (Lond.)* 574(Pt 2), 491–507.

Ito, M. (2008). Control of mental activities by internal models in the cerebellum. *Nat. Rev. Neurosci.* 9, 304–313.

Jantsch, H. H., Kemppainen, P., Ringler, R., Handwerker, H. O., and Forster, C. (2005). Cortical representation of experimental tooth pain in humans. *Pain* 18, 390–399.

Jung, P., Baumgärtner, U., Bauermann, T., Magerl, W., Gawehn, J., Stoeter, P., and Treede, R.-D. (2003). Asymmetry in the human primary somatosensory cortex and handedness. *Neuroimage* 19, 913–923.

Keller, T., Popovic, M. R., Pappas, I. P., and Muller, P. Y. (2002). Transcutaneous functional electrical stimulator “Compex Motion”. *Artif. Organs* 26, 219–223.

Kemppainen, P., Avellan, N. L., Handwerker, H. O., and Forster, C. (2003). Differences between tooth stimulation and capsaicin-induced neurogenic vasodilatation in human gingiva. *J. Dent. Res.* 82, 303–307.

Kenshalo, D. R. Jr., Chudler, E. H., Anton, F., and Dubner, R. (1988). SI nociceptive neurons participate in the encoding process by which monkeys perceive the intensity of noxious thermal stimulation. *Brain Res.* 454, 378–382.

Kulkarni, B., Bentley, D. E., Elliott, R., Youell, P., Watson, A., Derbyshire, S. W., Frackowiak, R. S., Friston, K. J., and Jones, A. K. (2005). Attention to pain localization and unpleasantness discriminates the functions of the medial and lateral pain systems. *Eur. J. Neurosci.* 21, 3133–3142.

Lancaster, J. L., Summerln, J. L., Rainey, L., Freitas, C. S., and Fox, P. T. (1997). The Talairach Daemon, a database server for Talairach atlas labels. *Neuroimage* 5, 633.

Lancaster, J. L., Woldorff, M. G., Parsons, L. M., Liotti, M., Freitas, C. S., Rainey, L., Kochunov, P. V., Nickerson, D., Mikiten, S. A., and Fox, P. T. (2000). Automated Talairach atlas labels for functional brain mapping. *Hum. Brain Mapp.* 10, 120–131.

Legrain, V., van Damme, S., Eccleston, C., Davis, K. D., Seminowicz, D. A., and Crombez, G. (2009). A neurocognitive model of attention to pain: behavioral and neuroimaging evidence. *Pain* 144, 230–232.

Maldjian, J. A., Laurienti, P. J., Kraft, R. A., and Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage* 19, 1233–1239.

Manto, M.-U. (2006). On the cerebello-cerebral interactions. *Cerebellum* 5, 286–288.

Mouraux, A., and Iannetti, G. D. (2009). Nociceptive laser-evoked brain potentials do not reflect nociceptive-specific neural activity. *J. Neurophysiol.* 101, 3258–3269.

Neugebauer, V., and Li, W. (2002). Processing of nociceptive mechanical and thermal information in central amygdala neurons with knee-joint input. *J. Neurophysiol.* 87, 103–112.

Neugebauer, V., and Li, W. (2003). Differential sensitization of amygdala neurons to afferent inputs in a model of arthritic pain. *J. Neurophysiol.* 89, 716–727.

Neugebauer, V., Li, W., Bird, G. C., and Han, J. S. (2004). The amygdala and persistent pain. *Neuroscientist* 10, 221–234.

Patrick, G. W., and Robinson, M. A. (1987). Collateral projections from trigeminal sensory nuclei to ventrobasal thalamus and cerebellar cortex in rats. *J. Morphol.* 192, 229–236.

Petrovic, P., Petersson, K. M., Ghatan, P. H., Stone-Elander, S., and Ingvar, M. (2000). Pain-related cerebral activation is altered by a distracting cognitive task. *Pain* 85, 19–30.

Peyron, R., Kupers, R., Jehl, J. L., Garcia-Larrea, L., Convers, P., Barral, F. G., and Laurent, B. (2007). Central representation of the RIII flexion reflex associated with overt motor reaction: an fMRI study. *Clin. Neurophysiol.* 37, 249–259.

Peyron, R., Laurent, B., and Garcia-Larrea, L. (2000). Functional imaging of brain responses to pain: a review and meta-analysis (2000). *Neurophysiol. Clin.* 30, 263–288.

Phan, K. L., Taylor, S. F., Welsh, R. C., Ho, S.-H., Britton, J. C., and Liberzon, I. (2004). Neural correlates of individual ratings of emotional salience: a trial-related fMRI study. *Neuroimage* 21, 768–780.

Ploghaus, A., Tracey, I., Clare, S., Gati, J. S., Rawlins, J. N., and Matthews, P. M. (2000). Learning about pain: the neural substrate of the prediction error for aversive events. *Proc. Natl. Acad. Sci. U.S.A.* 97, 9281–9286.

Rempel-Clower, N. L. (2007). Role of orbitofrontal cortex connections in emotion. *Ann. N. Y. Acad. Sci.* 1121, 72–86.

Saab, C. Y., Kawasaki, M., Al-Chaer, E. D., and Willis, W. D. (2001). Cerebellar cortical stimulation increases spinal visceral nociceptive responses. *J. Neurophysiol.* 85, 2359–2363.

Saab, C. Y., and Willis, W. D. (2003). The cerebellum: organization, functions and its role in nociception. *Brain Res.* 42, 85–95.

Schlereth, T., Baumgärtner, U., Magerl, W., Stoeter, P., and Treede, R.-D. (2003). Left-hemisphere dominance in early nociceptive processing in the human parasylvian cortex. *Neuroimage* 20, 441–454.

Schmahmann, J. D., and Caplan, D. (2006). Cognition, emotion and the cerebellum. *Brain* 129(Pt 2), 290–292.

Schmahmann, J. D., and Pandya, D. N. (1997). The cerebrocerebellar system. *Int. Rev. Neurobiol.* 41, 31–60.

Schoedel, A. L., Zimmermann, K., Handwerker, H. O., and Forster, C. (2008). The influence of simultaneous ratings on cortical BOLD effects during painful and non-painful stimulation. *Pain* 135, 131–141.

Scott, D. J., Heitzeg, M. M., Koeppe, R. A., Stohler, C. S., and Zubieta, J. K. (2006). Variations in the human pain stress experience mediated by ventral and dorsal basal ganglia dopamine activity. *J. Neurosci.* 26, 10789–10795.

Serrien, D. J., Ivry, R. B., and Swinnen, S. P. (2006). Dynamics of hemispheric specialization and integration in the context of motor control. *Nat. Rev. Neurosci.* 7, 160–166.

Sessle, B. J. (2000). Acute and chronic craniofacial pain: brainstem mechanisms of nociceptive transmission and neuroplasticity, and their clinical correlates. *Crit. Rev. Oral Biol. Med.* 11, 57–91.

Snyder, R. L., Faull, R. L., and Mehler, W. R. (1978). A comparative study of the neurons of origin of the spinocerebellar afferents in the rat, cat and squirrel monkey based on the retrograde transport of horseradish peroxidase. *J. Comp. Neurol.* 181, 833–852.

Strange, B. A., and Dolan, R. J. (2006). Anterior medial temporal lobe in human cognition: memory for fear and the unexpected. *Cogn. Neuropsychiatry* 11, 198–218.

Symonds, L. L., Gordon, N. S., Bixby, J. C., and Mande, M. M. (2006). Right-lateralized pain processing in the human cortex: an fMRI study. *J. Neurophysiol.* 95, 3823–3830.

Tracey, I., and Mantyh, P. W. (2007). The cerebral signature for pain perception and its modulation. *Neuron* 55, 377–391.

Treede, R. D., Kenshalo, D. R., Gracely, R. H., and Jones, A. K. (1999). The cortical representation of pain. *Pain* 79, 105–111.

Varnavas, G. G., and Grand, W. (1999). The insular cortex: morphological and vascular anatomic characteristics. *Neurosurgery* 44, 127–136; discussion 136–138.

Vogt, B. A. (2005). Pain and emotion interactions in subregions of the cingulate gyrus. *Nat. Rev. Neurosci.* 6, 533–544.

Watkins, L. R., Wiertelak, E. P., McGorry, M., Martinez, J., Schwartz, B., Sisk, D., and Maier, S. F. (1998). Neurocircuitry of conditioned inhibition of analgesia: effects of amygdala, dorsal raphe, ventral medullary, and spinal cord lesions on antianalgesia in the rat. *Behav. Neurosci.* 112, 360–378.

Wiech, K., Kalisch, R., Weiskopf, N., Pleger, B., Stephan, K. E., and Dolan, R. J. (2006). Anterolateral prefrontal cortex mediates the analgesic effect of expected and perceived control over pain. *J. Neurosci.* 26, 11501–11509.

Wiesenfeld-Hallin, Z. (2005). Sex differences in pain perception. *Gend. Med.* 2, 137–145.

Worsley, K. J., Marrett, S., Neelin, P., Vandal, A. C., Friston, K. J., and Evans, A. C. (1996). A unified statistical approach for determining significant signals in images of cerebral activation. *Hum. Brain Mapp*. 4, 58–73.

Youell, P. D., Wise, R. G., Bentley, D. E., Dickinson, M. R., King, T. A., Tracey, I., and Jones, A. K. (2004). Lateralisation of nociceptive processing in the human brain: a functional magnetic resonance imaging study. *Neuroimage* 23, 1068–1077.

7.3 STUDY 3

Where does it hurt most?

Tracing toothache intensity in the brain

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Submitted to:

The Journal of Dental Research

ABSTRACT

Identification of brain regions that differentially respond to pain intensity may improve our understanding of inexplicable odontalgia. This report analyzed cortical responses to painless and painful electric stimulation of a right human maxillary canine tooth. Functional magnetic resonance images were obtained during application of five graded stimulus strengths, ranging from below, at and above the individually determined pain thresholds. Subjects reported on a visual rating scale each stimulus with respect to evoked sensation. Based on hemodynamic responses of all pooled stimuli, a cerebral network was identified that largely corresponds to the known lateral and medial nociceptive system. Further analysis of the five graded stimulus strengths revealed positive linear correlations for the anterior insula bilaterally, the left anterior mid-cingulate as well as left pregenual cingulate cortices. Cerebral toothache intensity coding on a group level can thus be attributed to specific subregions within the cortical pain network.

KEY WORDS

facial pain/physiopathology, toothache/physiopathology, Physical Stimulation, Pain/physiopathology, physical stimulation, linear models, functional MRI

Running title: tracing toothache intensity in the brain

INTRODUCTION

Investigating human cerebral processes related to orofacial pain and specifically to toothache by functional neuroimaging is a relatively new direction in dental neuroscience (DaSilva et al., 2002; Ettlin et al., 2009; Weigelt et al., 2010; Brügger et al., 2011). Considering that the perceived intensity of a toothache is often inexplicable by the clinical dental status (Clark, 2006), more knowledge is required on central processes accounting for coding the strength of dental stimuli. Human experiments outside the orofacial region addressed this topic by applying stimuli of varying temperatures to the hand and measuring brain responses with positron emission tomography (PET) (Coghill et al., 1999)or functional magnetic resonance imaging (fMRI) (Lui et al., 2008). Further investigations applied laser stimuli to the hand and analyzed resulting brain activation with fMRI (Bornhövd et al., 2002; Buchel et al., 2002). The evidence established by stimulating these spinal nerve territories points towards specific cortical regions that code for stimulus intensity. These include the primary (SI) and secondary (SII) cortex, the anterior and posterior insula, and different subdivisions of the cingulate cortex (CC). Only one study compared human brain responses to weak and strong electric noxious dental pain stimuli (Jantsch et al., 2005). This study revealed in some but not all differential activity in the ipsilateral (right) medial insular cortex and in the left anterior and posterior cingulate cortex. The current report aimed at expanding previous findings by exploring cortical responses to five levels of electric dental stimulus strength, ranging from non-noxious to painful. We hypothesized to find stimulus strength dependent brain activation within SI, SII (parietal operculum), subdivisions of the insular and cingulate cortices and possibly the amygdala.

MATERIAL AND METHODS

PARTICIPANTS

13 right handed male subjects (age 22 - 49, mean 33.6) with no dental pain experience during the preceding year participated. Pathologies of test teeth were excluded by professional dental and radiographic examination. The study paradigm was approved by the local ethics committee and was conducted according to the guidelines of the Declaration of Helsinki. Participating subjects were financially compensated.

EXPERIMENTAL MATERIAL

Maxillary acrylic splints were fabricated from alginate impressions. Stainless steel electrodes were embedded in each splint at the labial and palatal center of the right upper canine. To minimize electric resistance, a 3mm round piece of hydrogel (AMGEL Technologies, AG 602-6, 8520 Lystrup, Denmark) was placed on the electrodes which were then covered by a thin layer of toothpaste (Signal Microgranuli, Unilever, Zug, Switzerland). Care was taken that splints did not evoke pain or discomfort. Electric stimulation was performed by the portable system Compex Motion (Keller et al., 2002). The presentation software (www.neurobs.com/presentation) controlled the experimental protocol. Shielded wires were used to avoid radiofrequency contamination by the stimulation current.

PSYCHOPHYSICAL TESTING

A test session was conducted to familiarize subjects with the stimulus within two weeks prior to the MR experiment. The scanner environment was simulated by dimming room light and subjects wore a headset playing a fMRI-EPI-sequence audiofile. They were instructed to close their eyes and concentrate explicitly on perceived stimulus intensity. The electrode holding splint was inserted and after 10 minutes adaptation, biphasic and bipolar direct currents pulses of 1ms duration were applied to the right upper canine for threshold determination. For stimulus detection threshold (SDT) and pain detection threshold (PDT), stimuli were applied according to the method of limits by Fechner (for details, see supplemental material figure 1). Pain tolerance threshold (PTT) was determined by increasing the stimulus current.

FMRI PROTOCOL

Subjects underwent the fMRI protocol in a Philips 3-Tesla Achieva System (Philips Medical System, Best, The Netherlands). First, individual SDT and PDT were retested. Since investigations indicated an association between diurnal rhythm and somesthesic perceptions (Fillingim and Ness, 2000), current investigation took place at the same daytime as the psychophysical examination.

The fMRI stimulation protocol consisted of 150 stimuli randomly applied at the following five stimulus strengths (30 stimuli/strength): PDT-40%, PDT-10%, PDT+20%, PDT+40% and PDT+60%. The interstimulus interval was randomized between 7.5 and 12.5 seconds (supplemental figure 2). Subjects were instructed to rate every stimulus on a computerized visual rating scale (coVRS) with 12 marks. The left ancor (first mark) was labeled “no sensation”, the 4^{th} mark “pain threshold” and the right ancor (12^{th} mark) “worst imaginable pain”. This approach enabled subjects to rate the perceived intensity of two painless and three painful stimuli (Fig. 3 supplemental material). It was reiterated that subjects focus on perceived stimulus intensity. The coVRS appeared one second after stimulus-delivery and was shown for six seconds and was then replaced by a fixation cross. After the experimental protocol, participants were asked whether they had perceived the stimulation in the test tooth only or also in adjacent tissue.

For the functional MR scans, a blood oxygen level dependent (BOLD) sensitive single-shot gradient echo planar imaging sequence was used to acquire 33 axial whole brain slices, using an 8 channel receive-only head coil. Parameters: echo time=30 ms, flip angle=75 degrees, repetition time=2500 ms, slice thickness=4 mm, inter-slice gap=0 mm, field of view=220 mm and matrix size in plane=128x128, resulting in a voxel size of 1.72x1.72x4 mm^3. Three dummy scans were first acquired and discarded to reach steady state magnetization. 180 high-resolution T1 weighted axial slices (spoiled gradient echo) were acquired with TR=20ms, flip angle=20°, voxel size=0.98x0.98x1.02 mm^3, FOV=24 cm, matrix=256x192, which were used as an underlay for individual functional maps.

DATA ANALYSIS

Psychophysical data (the relation between physical stimulus strength and perceived intensity rating) and data containing the relation between the physical stimulus strength and BOLD signal increase in each ROI were analyzed using SPSS 16 (SPSS Inc, Chicago, Illinois 60606, USA). SPM5 (http://www.fil.ion.ucl.ac.uk/spm) software package running on MatLab R2008b (Mathworks, Natiek, USA) was used for functional image analysis. In a first step, spatial realignment and reslicing to the first image in the series as reference was performed and it was assured that detected movement did not exceed 1.5 mm (translational) or 1° (rotational) in relation to the first image in the series. For studying group effects, data were normalized to the Montreal neurological institute (MNI) template brain followed by smoothing with a Gaussian kernel of 6 mm (FWHM). Image analysis for detection of significant BOLD signal changes in response to the five stimulus strengths (conditions) was performed on each subject's data by means of a fixed-effects model using the hemodynamic response function (implemented in SPM). Statistical parametric maps were calculated, yielding the significance of the model fit for each subject and condition. Average group statistical maps were then calculated using second level one-sample t-tests for every stimulus strength. Voxel T-values, corresponding to significant stimulus related increase were color-coded and superimposed onto the MNI single-subject-T1 brain. For a more detailed regional investigation of trigeminal intensity coding, we chose to calculate the mean activation in predefined anatomical regions of interests (ROI) with images provided by the "WFU Pickatlas" (Maldjian et al., 2003) and the anatomy toolbox (Eickhoff et al., 2005). Our approach predominantly focused on brain areas known from general pain literature reviews (Peyron et al., 2000; Apkarian et al., 2005; Farrell et al., 2005) as well as from literature specifically addressing stimulus intensity coding (Coghill et al., 1999; Bornhövd et al., 2002; Buchel et al., 2002; Baliki et al., 2009). ROIs were: postcentral gyrus SI, secondary somatosensory gyrus SII (parietal operculum from the anatomy toolbox) and the amygdala. Further, the "insula-ROI" provided by the WFU-Pickatlas was divided into three subdivisions (anterior (aIC), medial (mIC) and posterior (pIC) according to Brooks (Brooks et al., 2002) since several reports suggest differential anatomical (Varnavas and Grand, 1999) and functional (Kurth et al., 2010) contributions of these insular subdivisions to stimulus processing. To account for the functional complexity of the cingulate cortex, we also subdivided this structure based on (Vogt, 2005) and analyzed the ROIs anterior mid-cingulate

cortex (aMCC) and the pregenual cingulate cortex (pgACC) (Vogt, 2005). The numbers of activated voxels as well as mean and maximum activation within each ROI were calculated for all five stimulus strengths. A repeated measures ANOVA was calculated across all ROIs with stimulus strength as “within subject factor”. Linear contrasts were tested in order to assess the relation between mean ROI activation (T-values) and administered stimuli. Intensity coding was defined as the linear correlation between increasing "stimulus strength" and intensifying BOLD signal strength. The exact relation was defined as polynomial within contrast.

RESULTS

PSYCHOPHYSICS

All volunteers perceived the sensation in the stimulated tooth only and not in adjacent teeth or tissues. Further, volunteers reported no lingering sensation after the stimulation, indicating that no sensitization had been induced with our experimental setup. The average stimulation current and corresponding coVAS ratings are illustrated in Figure 1.

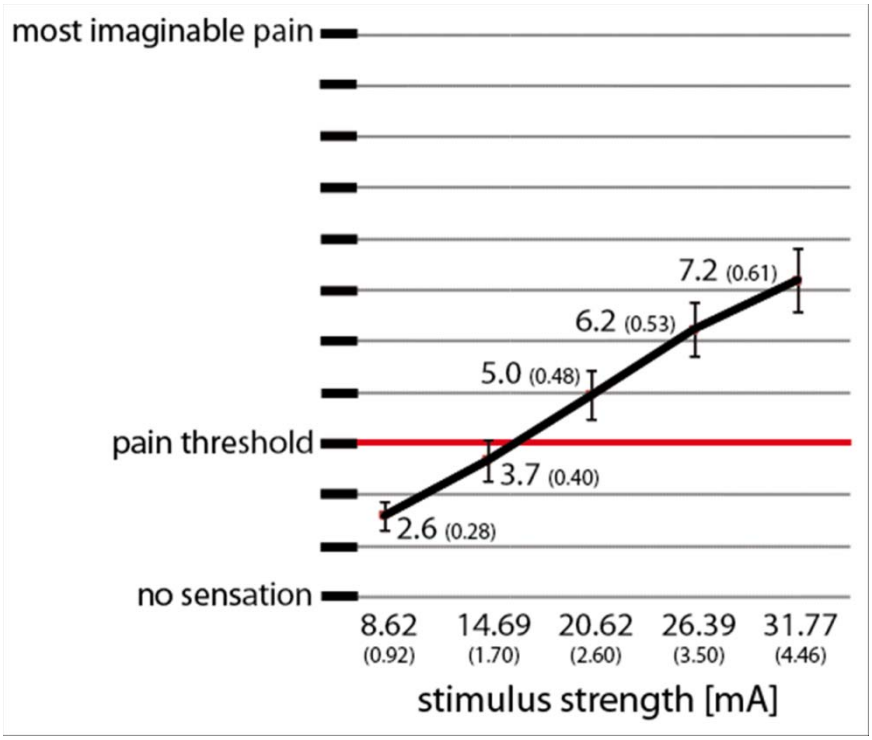


Figure 1: Group mean stimulus current in mA and corresponding mean coVAS ratings (with standard errors of the mean in brackets and graphically shown as T-bars) during the fMRI stimulation experiment. The pain threshold is illustrated by the red line.

The correlations for each individual were: r_1=0.819, r_2=0.844, r_3=0.613, r_4=0.920, r_5=0.837, r_6=0.786, r_7=0.804, r_8=0.839, r_9=0.635, r_{10}=0.846, r_{11}=0.525, r_{12}=0.870, r_{13}=0.587. All correlations were significant with $p<0.001$. The mean of the individual correlations was r=0.763.

Brain Response Analysis

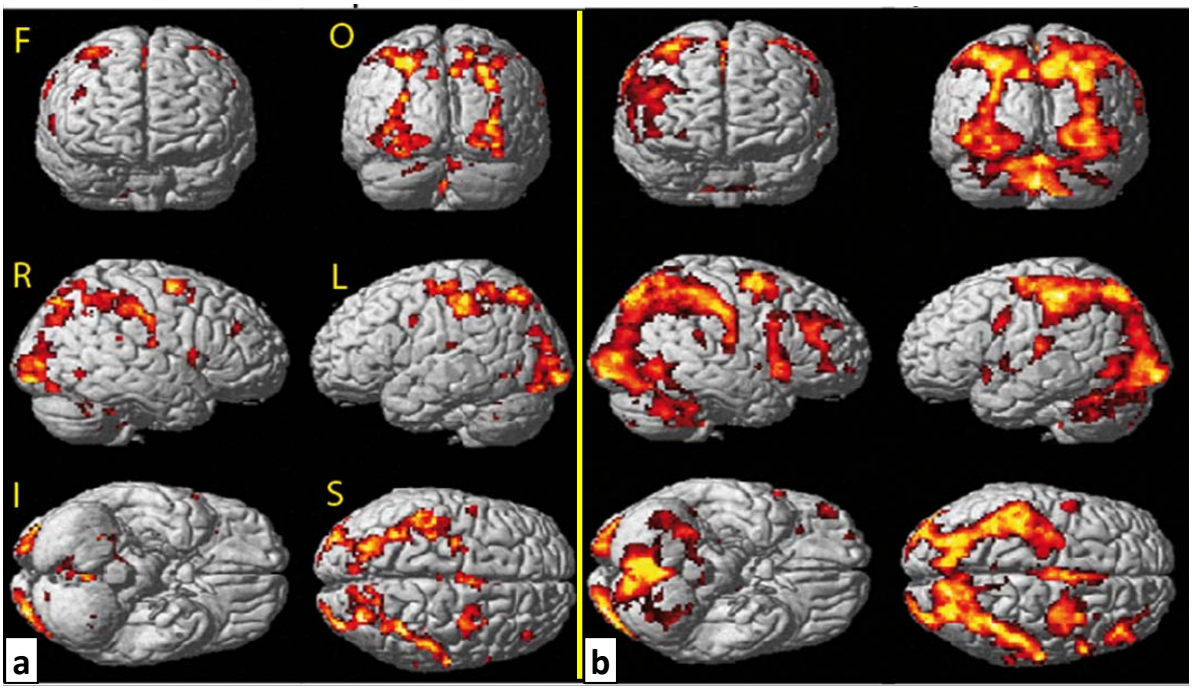


Figure 2: fMRI activation projected on the rendered MNI single subject T1 template. Illustrated is the brain activity in response to the pooled painless (a) and painful (b) stimulus strengths revealed by one sample t tests. The statistical threshold was conservative: FWE-corrected with $p < 0.05$ and an extent threshold of 10 voxel. F = frontal, O = occipital, R = right, L = left, I = inferior and S = superior.

Tooth stimulation was associated with significantly increased brain activity across a bilateral distributed network including SI, SII, motor/premotor and supplementary motor areas, superior temporal and midtemporal, parietal and occipital areas, the cerebellum as well as superior and inferior frontal areas. Further activation was found in the cingulate and insular cortex subdivisions as well as in subcortical brain structures like thalamus, putamen and brainstem. Note that all stimuli whether painless or painful generally induced increased BOLD responses in analogous brain regions. However, painful stimuli resulted in larger clusters and higher p and t-values, respectively (suppl. table 1).

REGION OF INTEREST (ROI) ANALYSIS

A significant main effect of stimulus strength on BOLD signal was observed in the following areas: bilateral aIC (left: F=2.793, p=0.036 and right: F=3.481, p=0.014) as well as left pgACC (F=2.676, p=0.043). These three brain regions also showed a significant linear contrast (in the sequence as above: F=4.528, p=0.055; F=8.46, p=0.013; F=6.717, p=0.024). Further, a trend for intensity coding was detected in the left aMCC (F=4.012, p=0.068; linear contrast: F=4.327, p=0.060). Other regions investigated revealed no intensity coding properties, namely SI, SII (parietal operculum) and amygdala. Right aMCC and pgACC neither demonstrated significant main effects nor linear contrasts (Fig. 3).

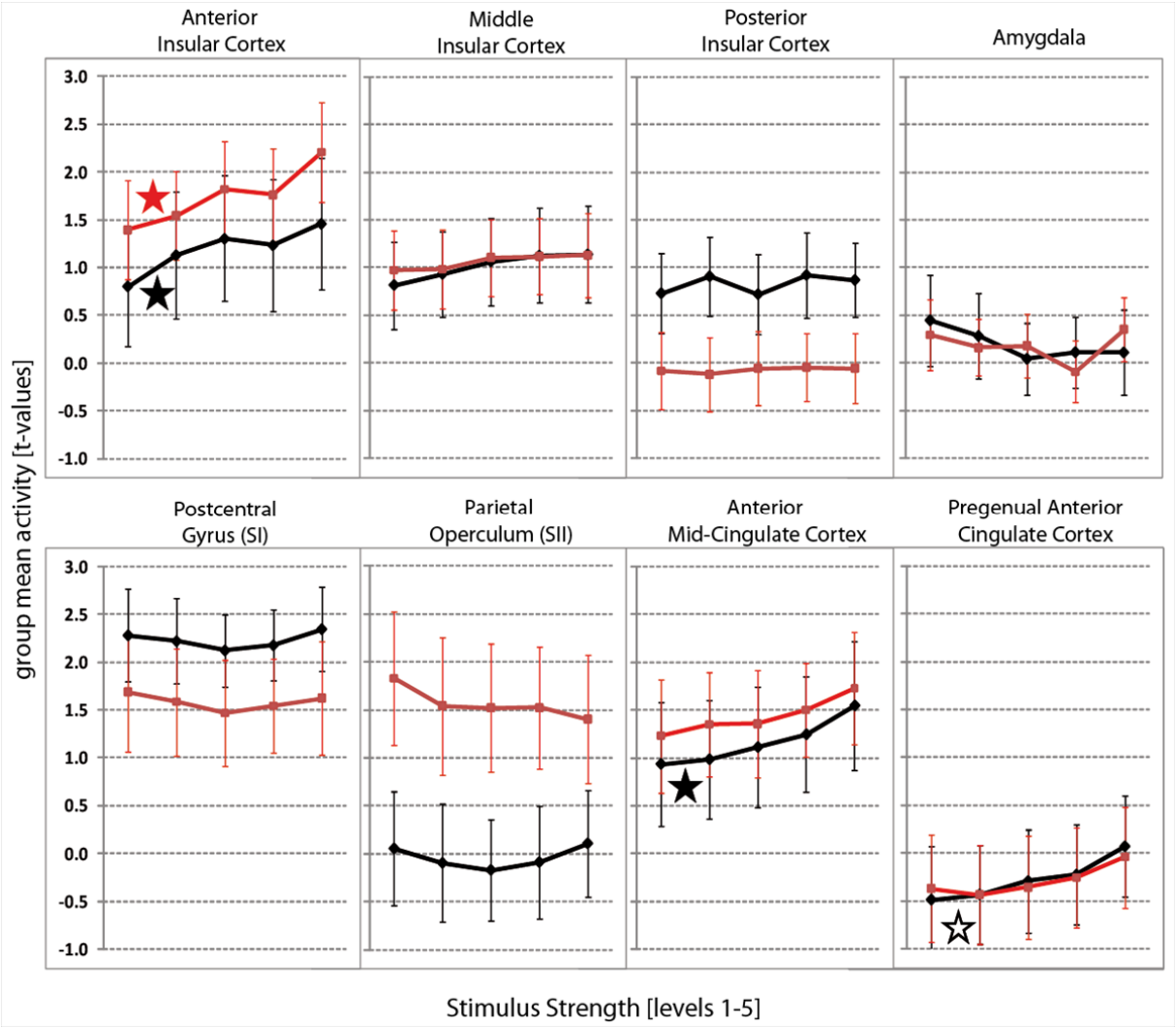


Figure 3: Results of the ROI analysis. Illustrated is the relationship of increasing stimulus strength (x-axis) and mean group signal activity (y-axis). Stars indicate regions demonstrating significant (filled) or trend-like (empty) intensity coding. Red and black lines represent right (ipsilateral) and left (contralateral) hemispheric ROIs, respectively. T Bars reflect standard errors of the mean.

DISCUSSION

The key finding of this study is that electric dental stimuli of graded current amplitude evoked corresponding BOLD signal changes in the aIC bilaterally and in two subdivisions of the left cingulate cortex. These brain regions therefore appear relevant for coding intensity perception of the physical stimulus strengths. We arrived at this finding by stimulating the right upper canine with five graded electric stimulus strengths ranging from painless to moderately painful. coVRS intensity ratings were obtained for every stimulus and cortical activations were analyzed for whole brain and for predefined ROIs. Our paradigm demonstrates that stimuli originating in a single tooth generally activate - in a intensity dependant mode - a bilateral brain network analogous to bodily stimuli. This activation patterns confirms our previous observations from bilateral dental stimulation (Brügger et al., in press). A more detailed analysis focusing on specific ROI revealed both similarities and differences in comparison to graded thermal stimulation of the hand (Buchel et al., 2002; Bornhövd et al., 2002). Below, we discuss the findings in more detail of those ROIs we hypothesized to potentially code for intensity.

Insular Cortex

The IC is robustly activated in response to a wide variety of noxious and innocuous stimuli, and is therefore considered a multimodal integration structure (Augustine, 1996; Craig, 2003). Still, the functional specificity of its subregions remains subject of continuous discussion (Kurth et al., 2010). Baliki et al. recently identified bilateral localized activity within the aIC that correlated with the magnitude of visual stimuli (mag-INS) and in close proximity a region that specifically coded for pain levels (noci-INS) (Baliki et al., 2009). Our findings confirm the bilateral aIC involvement in pain magnitude estimation and expand its function to embrace also painless stimuli. Only one report previously compared weak vs. strong electric toothpain and detected intensity coding in more posterior IC regions (ipsilateral mIC/pIC and contralateral mIC) (Jantsch et al., 2005). Considering that our stimulus paradigm induced measurable metabolic changes in the IC in another study (Gutzeit et al., 2010), it seems justified to focus on the role of IC subregions in dental somatosensory and pain processing in the future.

Cingulate Cortex (CC)

A comparison of CC findings between studies is hampered by the different approaches of subdividing this large and functionally complex brain structure. For analysis of our data we followed the terminology suggestions by Vogt, 2005. Electric dental stimulation elicited neuronal activation in clusters covering aMCC and pMCC and to a lesser extent the pgACC (fig. 3, suppl. table 1). Among these, contralateral (left) aMCC and pgACC revealed linear BOLD signal changes correlating to physical stimulus strength (fig. 3). Generally, the aMCC divisions are more commonly associated with cognitive-evaluative aspects whereas the pgACC has been linked to emotional and/or affective dimensions of the stimulus perception (Vogt, 2005). It seems intuitive that more intense stimuli prompt higher arousal, stronger emotions and enhanced response evaluation. Evidence for the role of the ACC in affective magnitude rating has existed for some time (Rainville et al., 1999). More recently, (Buchel et al., 2002) and (Lui et al., 2008) differentially analyzed perception coding of graded stimulus strengths. Both groups found robust BOLD signal alterations in aMCC and pgACC. Noxious dental stimuli applied in a previous study also resulted in aMCC and pgACC activation (Jantsch et al., 2005). In summary, evidence is converging that anterior parts of the CC play a pivotal role in coding intensity of spinal and dental stimuli.

Regions not demonstrating significant intensity coding effect

SI and SII revealed strong bilateral hemodynamic responses, yet no linear relationship between stimulus strength and brain activity was detected in either region (Fig 3). The main function assigned to SI is somatotopic stimulus encoding (Peyron et al., 2000; Apkarian et al., 2005). Although intensity related activity has been described (e.g., Kenshalo et al., 2000; Moulton et al., 2005), the Baliki report specifically designed to investigate magnitude estimation observed no relation between pain intensity and SI activity (Baliki et al., 2009). SII is a brain region most consistently activated by pain (Peyron et al., 2000; Apkarian et al., 2005). Based upon recent cytoarchitectural classification (Eickhoff et al., 2006b), activity resulting from both, noxious and non-noxious stimuli can be allocated to specific SII subregions termed operculum 1 (OP1) to OP4. Pain related activity is preferentially located within OP1 (Coghill et al., 1999), (Bornhövd et al., 2002); (Porro, 2003)), whereas painless stimuli preferentially evoke more anterior activation at the border between OP1 and OP4 (Eickhoff et al., 2006a; Burton et al., 2008). The finding by (Jantsch et al., 2005) of greater

BOLD signal changes in SI/SII during strong compared to weak toothpain could not be reproduced in our study using five stimulus strengths.

Limitations of the study

It needs to be bared in mind that perceptions in the context of an experiment do not necessarily reflect clinical experiences. Although we asked volunteers to selectively focus on perceived stimulus intensity, other psychological parameters such as anticipation, unpleasantness or suffering may also be reflected in the brain activation patterns observed. Yet, attempting to comprehensively monitor all these aspects is unfeasible due to experimental time constraints.

CONCLUSION

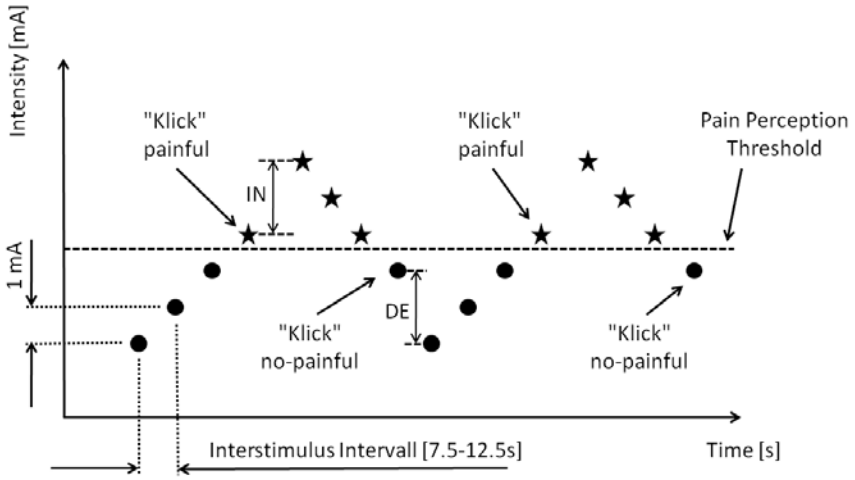
Our study confirms that no single cortical "magnitude estimator" exists, but rather a group of specific brain areas preferentially code for the perceived intensity of stimulus strength, namely the aIC bilaterally as well as left aMCC and pgACC. Our neuroimaging data thus support current concepts which consider modular networks an important organizational principle of human brain architecture (Meunier et al., 2010). If future investigations confirm the robustness of our results, differential activity in these brain regions may potentially serve as supplemental outcome measure for dental analgesic interventions in the future.

ACKNOWLEDGMENTS

This study was financially supported by GlaxoSmithKline, Consumer Healthcare, Weybride, London.

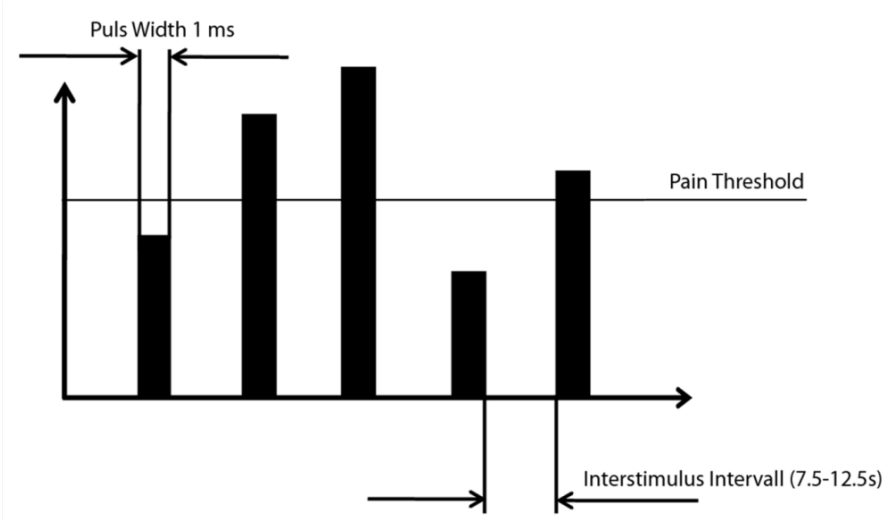
All authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

SUPPLEMENTAL MATERIAL



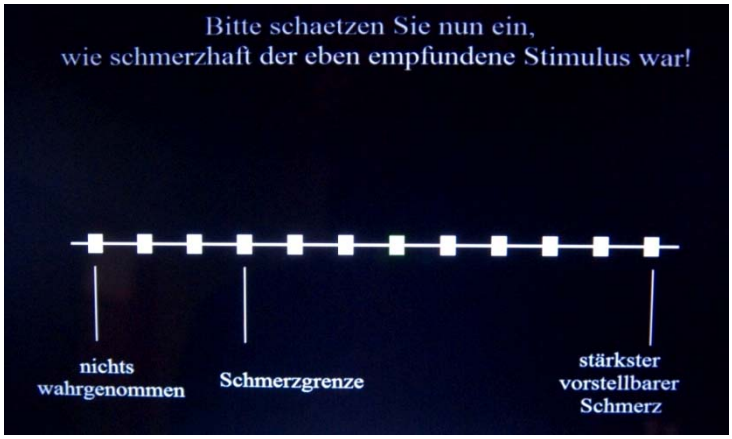
Suppl. Figure 1

Diagram of the "methods of limits" for the determination of Pain Detection Threshold (PDT). "Klick" means, that subjects had to push the mouse-button, when they felt the delivered stimulus as painful and again, when they felt the stimulus as clearly no-painful. The program automatically increased (IN) or decreased (DE) the applied stimulus-intensity after each "Klick", and the paradigm run again.



Suppl. Figure 2

Schematic of the fMRI paradigm. Stimlus duration were set to 1 ms, interstimulus intervals were kept between 7.5 and 12.5 seconds. Strength of the stimuli were PDT - 20%, PDT, PDT + 20%, PDT + 40% and PDT + 60%. The different strength have then been applied randomly and subjects were required to rate every stimulus with respect to their perceived intensity by means of a MR compatible rating scale (Fig. 3).



Suppl. Figure 3 illustrates the computerized visual rating scale (coVRS) the way it had been projected after every stimulus for 6 seconds. The white rectangle changed into green, when subjects rated their intensity-perception. Left; no perception (nichts wahrgenommen), the fourth rectangle; pain threshold (Schmerzgrenze), right; worst imaginable pain (stärkster vorstellbarer Schmerz). Important to note: subjects were trained prior to the fMRI experiment in handling the coVRS correctly and to answer questions/uncertainties. However, all of them understood quickly the use of the scale.

Suppl. Table 1

Anatomical location, coordinates and cluster size of the maximum t and p- values for painful stimulation vs. baseline.

Brain Region	MNI coordinates of local maxima x / y / z	Cluster Size (N Voxel)	T -value of local maxima	p-value corrected for multiple comparisions (FWE-cor)
L Postcentral Gyrus	-42 -36 52	37076	21.42	0.000
R Middle Occipital Gyrus	34 -88 2		20.77	0.000
L Parietal Inferior Gyrus	-28 -52 44		20.45	0.000
R Inferior Frontal Gyrus	58 10 8	3063	17.2	0.000
R Medial Frontal Gyrus	42 40 32		14.86	0.000
R Anterior Insula	34 22 2		14.71	0.000
R Superior Temporal Gyrus	66 -36 22	119	13.63	0.000
L Precentral Gyrus	-54 2 36	364	13.47	0.000
R Posterior Cingulate Gyrus	10 -38 26		10.78	0.000
L Thalamus	-16 -22 8	566	12.59	0.000
R Thalamus	12 -16 10	226	11.86	0.000
R Middle Insular Cortex	46 0 -2	116	10.17	0.000
L Putamen	-22 10 -4	60	9.74	0.000
L Middle Temporal Gyrus	-52 -48 6	60	8.79	0.000
L Middle Frontal Gyrus	-34 36 40	24	8.54	0.000
L Brainstem	-6 -16 -18	18	7.73	0.000
L Superior Frontal Gyrus	-32 50 30	27	7.68	0.000
R Middle Temporal Gyrus	48 -46 8	68	7.37	0.000
R Superior Frontal Gyrus	30 60 -8	10	6.81	0.000
L Inferior Frontal Gyrus	-48 36 16	15	6.33	0.002

REFERENCES

Apkarian AV, Bushnell MC, Treede RD, Zubieta JK (2005). Human brain mechanisms of pain perception and regulation in health and disease. European journal of pain (London, England) 9:463–484.

Augustine JR (1996). Circuitry and functional aspects of the insular lobe in primates including humans. Brain Res. Brain Res. Rev. 22:229–244.

Baliki MN, Geha PY, Apkarian AV (2009). Parsing pain perception between nociceptive representation and magnitude estimation. J. Neurophysiol. 101:875–887.

Bornhövd K, Quante M, Glauche V, Bromm B, Weiller C, Büchel C (2002). Painful stimuli evoke different stimulus-response functions in the amygdala, prefrontal, insula and somatosensory cortex: a single-trial fMRI study. Brain 125:1326–1336.

Brooks JC, Nurmikko TJ, Bimson WE, Singh KD, Roberts N (2002). fMRI of thermal pain. effects of stimulus laterality and attention. Neuroimage 15:293–301.

Brügger M, Ettlin DA, Meier ML, Keller T, Luechinger R, Barlow A et al. (2011). Taking sides with pain - Lateralization aspects related to cerebral processing of dental pain. Front. Hum. Neurosci. 5:12. doi: 10.3389/fnhum.2011.00012.

Buchel C, Bornhovd K, Quante M, Glauche V, Bromm B, Weiller C (2002). Dissociable neural responses related to pain intensity, stimulus intensity, and stimulus awareness within the anterior cingulate cortex. a parametric single-trial laser functional magnetic resonance imaging study. J Neurosci 22:970–976.

Burton H, Sinclair RJ, McLaren DG (2008). Cortical network for vibrotactile attention: a fMRI study. Hum Brain Mapp 29:207–221.

Clark GT (2006). Persistent orodental pain, atypical odontalgia, and phantom tooth pain: when are they neuropathic disorders? J Calif Dent Assoc 34:599–609.

Coghill RC, Sang CN, Maisog JM, Iadarola MJ (1999). Pain intensity processing within the human brain: a bilateral, distributed mechanism. J. Neurophysiol. 82:1934–1943.

Craig AD (2003). A new view of pain as a homeostatic emotion. Trends in neurosciences 26:303–307.

DaSilva AFM, Becerra L, Makris N, Strassman AM, Gonzalez RG, Geatrakis N et al. (2002). Somatotopic activation in the human trigeminal pain pathway. J. Neurosci. 22:8183–8192.

Eickhoff SB, Amunts K, Mohlberg H, Zilles K (2006a). The human parietal operculum. II. Stereotaxic maps and correlation with functional imaging results. Cereb. Cortex 16:268–279.

Eickhoff SB, Schleicher A, Zilles K, Amunts K (2006b). The human parietal operculum. I. Cytoarchitectonic mapping of subdivisions. Cereb. Cortex 16:254–267.

Eickhoff SB, Stephan KE, Mohlberg H, Grefkes C, Fink GR, Amunts K et al. (2005). A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. Neuroimage 25:1325–1335.

Ettlin DA, Brügger M, Keller T, Luechinger R, Jäncke L, Palla S et al. (2009). Interindividual differences in the perception of dental stimulation and related brain activity. Eur. J. Oral Sci. 117:27–33.

Farrell MJ, Laird AR, Egan GF (2005). Brain activity associated with painfully hot stimuli applied to the upper limb. a meta-analysis. Human brain mapping 25:129–139.

Fillingim RB, Ness TJ (2000). Sex-related hormonal influences on pain and analgesic responses. Neuroscience and biobehavioral reviews 24:485–501.

Gutzeit A, Meier D, Meier ML, Weymarn C von, Ettlin DA, Graf N et al. (2010). Insula-specific responses induced by dental pain. A proton magnetic resonance spectroscopy study. Eur Radiol. doi:10.1007/s00330-010-1971-8.

Jantsch HH, Kemppainen P, Ringler R, Handwerker HO, Forster C (2005). Cortical representation of experimental tooth pain in humans. Pain 118:390–399.

Keller T, Popovic MR, Pappas IP, Muller PY (2002). Transcutaneous functional electrical stimulator "Compex Motion". Artificial organs 26:219–223.

Kenshalo DR, Iwata K, Sholas M, Thomas DA (2000). Response properties and organization of nociceptive neurons in area 1 of monkey primary somatosensory cortex. J. Neurophysiol. 84:719–729.

Kurth F, Zilles K, Fox PT, Laird AR, Eickhoff SB (2010). A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. Brain Struct Funct 214:519–534.

Lui F, Duzzi D, Corradini M, Serafini M, Baraldi P, Porro CA (2008). Touch or pain? Spatio-temporal patterns of cortical fMRI activity following brief mechanical stimuli. Pain 138:362–374.

Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage 19:1233–1239.

Meunier D, Lambiotte R, Bullmore ET (2010). Modular and hierarchically modular organization of brain networks. Front Neurosci 4:200.

Moulton EA, Keaser ML, Gullapalli RP, Greenspan JD (2005). Regional intensive and temporal patterns of functional MRI activation distinguishing noxious and innocuous contact heat. J. Neurophysiol. 93:2183–2193.

Peyron R, Laurent B, García-Larrea L (2000). Functional imaging of brain responses to pain. A review and meta-analysis (2000). Neurophysiologie clinique = Clinical neurophysiology 30:263–288.

Porro CA (2003). Functional imaging and pain: behavior, perception, and modulation. Neuroscientist 9:354–369.

Rainville P, Carrier B, Hofbauer RK, Bushnell MC, Duncan GH (1999). Dissociation of sensory and affective dimensions of pain using hypnotic modulation. Pain 82:159–171.

Varnavas GG, Grand W (1999). The insular cortex. morphological and vascular anatomic characteristics. Neurosurgery 44:127-36; discussion 136-8.

Vogt BA (2005). Pain and emotion interactions in subregions of the cingulate gyrus. Nature reviews 6:533–544.

Weigelt A, Terekhin P, Kemppainen P, Dörfler A, Forster C (2010). The representation of experimental tooth pain from upper and lower jaws in the human trigeminal pathway. Pain 149:529–538.

8. GENERAL DISCUSSION

The findings of each experiment have been extensively discussed in the specific discussion sections of the manuscripts. The following chapter will therefore focus on issues concerning more comprehensive aspects addressed in the studies as well as incorporating a wider perspective regarding our findings.

Many studies already investigated cortical processes of spinally induced nociception, but almost nothing had been published with the focus on pain derived from the human trigeminal somatosensory system. This thesis intended to close this gap by providing respective data concerning the question: how the brain masters trigeminal mediated somatosensory input as well as specifically different facets of tooth pain.

With experiment 1, we primarily aimed at establishing an fMRI compatible stimulation paradigm to induce dental perceptions from barely perceivable till slightly painful. The experience derived from this work helped enormously for two important reasons: In this study, we underestimated the stringent necessity of an extensive psychophysical testing prior to the effective fMRI experiment. We copied this "bad-habit" from the literature where mostly, the volunteers are "familiarized" with the stimulation setup immediately before the experiment starts. Perhaps, this is possible in some rationales but it's clearly not advisable in pain studies, as the danger to provoke many other influencing side-effects like anxiety, agitation or general feelings of unpleasantness is most likely. All of them have a high potential of biasing the results, especially according to brain responses. The second reason, which in fact is colligated with the first one; there is simply more time to explain in detail what they have to expect and also address specific questions volunteers quite often have. However, despite these limitations of the first investigation, we were able to demonstrate the feasibility of the developed experimental setting and received a first impression of how the brain processes tooth related sensations. Due to both aspects, we were encouraged to go further in the project.

With experiment 2 and 3, we incorporated the lessons from study 1 in an exemplary manner as we conducted time consuming psychophysical pre-assessments in order to familiarize the volunteers and to derive the important psychophysical parameters. Within these assessment scenarios, all relevant parameters have been tested and determined like perception-, pain- and pain-tolerance thresholds as well as aspects concerning descriptive facets of the pain

experience. The derived results were really promising, as experiment 2 demonstrated a very robust cortical activation network, including all areas commonly described in the imaging related pain literature, generally termed the pain matrix. To examine the classical arrangement of a possible lateral and medial pain system (based on spinally induced cortical nociception), a region of interest analysis shed further light into specifics of trigeminal mediated response patterns. On this basis, we presented new evidence that the posterior insular cortex (pIC) is involved in stimulus encoding and that the anterior subdivision of the midcingulate cortex (aMCC) as well the perigenual subdivision of the anterior cingulate cortex (pACC) may have a very specific role according to the selection or inhibition of adequate pain responses. A further remarkable observation was that in contrast to many other pain studies, not only robust activation of the amygdala was elicited but additionally, significant contralateral activation dominance became apparent within this particular brain structure. Especially, this finding demonstrated for the first time in a human fMRI study that the amygdala may also encode somatotopic information that have only been described in animal related literature (Neugebauer and Li, 2002; Neugebauer et al., 2004).

Study 3 confirms suggestions of a magnitude estimation system within the neural pain circuitry; however with a remarkable extension that have not been described so far. We found that most likely not a single cortical magnitude estimator exists, but rather a group of specific brain areas, like the anterior insular cortex (aIC) and the anterior mid-cingulate cortex (aMCC) which seem to code preferentially for the perceived intensity of the administered stimulus strength. However, which of the areas are responsible for the consciousness of such an experience is to date not clear and highly debated. Therefore, future examinations have to be conducted in order to substantiate this particular finding by studying the respective brain structures with a higher resolution (7 Tesla) that allows a much more detailed acquisition and calculation of the related signals as well as to focus on the neural network architecture by calculating dynamic causal models that provide the information of how the different brain regions are functionally connected (Stephan and Friston, 2010). A still unresolved issue is related to the question, whether the presented results of this thesis are a unique feature for the trigeminal system or do they reflect a general property of the neuronal system under some specific pain conditions. This question has to be addressed and clarified by comparing cortical responses of spinally and trigeminally mediated somatosensory inputs with the same stimulation modality.

8.1 CONCLUSION

The present work closes the gap within the imaging related pain research by providing an option to study cortical and subcortical correlates of trigeminally mediated sensations and nociception and specifically tooth pain.

By conducting three experiments, we demonstrated that the brain network activated by experimentally induced tooth pain, is comparable to the commonly referred cortical pain matrix but with specific and interesting differences in some of the activated brain regions as the amygdala and subdivisions of insular and cingulate cortices. Because of this fact, we introduced the term *cortical dental pain matrix* to emphasize this possibly special role of the trigeminal system concerning somatosensory input to the human brain. Additionally, with the knowledge derived from the presented data, the prerequisites to examine the neural underpinnings of dentinal hypersensitivity have been established.

9. REFERENCES

Apkarian AV, Bushnell MC, Treede RD, Zubieta JK (2005). Human brain mechanisms of pain perception and regulation in health and disease. *European journal of pain (London, England)* 9:463–484.

Ash MM, Nelson SJ, Wheeler RC (2003). Wheeler's dental anatomy, physiology, and occlusion. [CD ROM inside]. 8th ed. /. Philadelphia: Saunders.

Baker LL, Chambers R, DeMuth SK, Villar F (1997). Effects of electrical stimulation on wound healing in patients with diabetic ulcers. *Diabetes Care* 20:405–412.

Baliki MN, Geha PY, Apkarian AV (2009). Parsing pain perception between nociceptive representation and magnitude estimation. *J. Neurophysiol.* 101:875–887.

Bartold PM (2006). Dentinal hypersensitivity: a review. *Australian dental journal* 51:212-8; quiz 276.

Basbaum AI, Bautista DM, Scherrer G, Julius D (2009). Cellular and molecular mechanisms of pain. *Cell* 139:267–284.

Basbaum AI, Julius D (2006). Toward better pain control. *Sci. Am.* 294:60–67.

Blickenstorfer A, Kleiser R, Keller T, Keisker B, Meyer M, Riener R et al. (2008). Cortical and subcortical correlates of functional electrical stimulation of wrist extensor and flexor muscles revealed by fMRI. *Human brain mapping*.

Broggi G (2008). Pain and psycho-affective disorders. *Neurosurgery* 62:901-19; discussion 919-20.

Brooks JC, Nurmikko TJ, Bimson WE, Singh KD, Roberts N (2002). fMRI of thermal pain. effects of stimulus laterality and attention. *NeuroImage* 15:293–301.

Brooks JC, Zambreanu L, Godinez A, Craig AD, Tracey I (2005). Somatotopic organisation of the human insula to painful heat studied with high resolution functional imaging. *NeuroImage* 27:201–209.

Buchel C, Bornhovd K, Quante M, Glauche V, Bromm B, Weiller C (2002). Dissociable neural responses related to pain intensity, stimulus intensity, and stimulus awareness within the anterior cingulate cortex. A parametric single-trial laser functional magnetic resonance imaging study. *J Neurosci* 22:970–976.

Buchel C, Dolan RJ, Armony JL, Friston KJ (1999). Amygdala-hippocampal involvement in human aversive trace conditioning revealed through event-related functional magnetic resonance imaging. *J Neurosci* 19:10869–10876.

Byers MR (1984). Dental sensory receptors. *Int. Rev. Neurobiol.* 25:39–94.

Byers MR, Närhi MV (2000). Dental injury models: experimental tools for understanding neuroinflammatory interactions and polymodal nociceptor functions. *Crit. Rev. Oral Biol. Med.* 10:4–39.

Cadden SW, Lisney SJ, Matthews B (1983). Thresholds to electrical stimulation of nerves in cat canine tooth-pulp with A beta-, A delta- and C-fibre conduction velocities. *Brain Res.* 261:31–41.

Casey KL (2000). Concepts of pain mechanisms: the contribution of functional imaging of the human brain. *Prog. Brain Res.* 129:277–287.

Chen AC (2008). Pain perception and its genesis in the human brain. *Sheng li xue bao : [Acta physiologica Sinica]* 60:677–685.

Coghill RC, Gilron I, Iadarola MJ (2001). Hemispheric lateralization of somatosensory processing. *J. Neurophysiol.* 85:2602–2612.

Craig AD, Zhang ET, Blomqvist A (1999). A distinct thermoreceptive subregion of lamina I in nucleus caudalis of the owl monkey. *J. Comp. Neurol.* 404:221–234.

Craig ADB (2003). Pain mechanisms: labeled lines versus convergence in central processing. *Annu. Rev. Neurosci.* 26:1–30.

Dababneh RH, Khouri AT, Addy M (1999). Dentine hypersensitivity - an enigma? A review of terminology, mechanisms, aetiology and management. *British dental journal* 187:606-11; discussion 603.

DeSantana JM, Walsh DM, Vance C, Rakel BA, Sluka KA (2008). Effectiveness of transcutaneous electrical nerve stimulation for treatment of hyperalgesia and pain. *Current rheumatology reports* 10:492–499.

Dubner R (1986). Pain control in dentistry: the anatomic and physiologic basis of orofacial pain. *The Compendium of continuing education in dentistry* 7:408, 410-6, 418.

Ettlin DA, Zhang H, Lutz K, Järmann T, Meier D, Gallo LM et al. (2004). Cortical activation resulting from painless vibrotactile dental stimulation measured by functional magnetic resonance imaging (FMRI). *J. Dent. Res.* 83:757–761.

Farrell MJ, Laird AR, Egan GF (2005). Brain activity associated with painfully hot stimuli applied to the upper limb. a meta-analysis. *Human brain mapping* 25:129–139.

Fitzek S, Fitzek C, Huonker R, Reichenbach JR, Mentzel H, Witte OW et al. (2004). Event-related fMRI with painful electrical stimulation of the trigeminal nerve. *Magnetic resonance imaging* 22:205–209.

Gondin J, Guette M, Ballay Y, Martin A (2005). Electromyostimulation training effects on neural drive and muscle architecture. *Medicine and science in sports and exercise* 37:1291–1299.

Henry Gray. 1918. Anatomy of the Human Body: Bibliographic Record. http://www.bartleby.com/br/107.html (Jan 10, 2009).

Iadarola MJ, Berman KF, Zeffiro TA, Byas-Smith MG, Gracely RH, Max MB et al. (1998). Neural activation during acute capsaicin-evoked pain and allodynia assessed with PET. *Brain* 121 (Pt 5):931–947.

Ikeda H, Suda H (2003). Sensory experiences in relation to pulpal nerve activation of human teeth in different age groups. *Arch. Oral Biol.* 48:835–841.

Jantsch HH, Kemppainen P, Ringler R, Handwerker HO, Forster C (2005). Cortical representation of experimental tooth pain in humans. *Pain* 118:390–399.

Keller T, Popovic MR, Pappas IP, Muller PY (2002). Transcutaneous functional electrical stimulator "Compex Motion". *Artificial organs* 26:219–223.

Keogh E, Herdenfeldt M (2002). Gender, coping and the perception of pain. *Pain* 97:195–201.

Khalil Z, Merhi M (2000). Effects of aging on neurogenic vasodilator responses evoked by transcutaneous electrical nerve stimulation: relevance to wound healing. *J. Gerontol. A Biol. Sci. Med. Sci.* 55:B257-63.

Klingberg G, Broberg AG (2007). Dental fear/anxiety and dental behaviour management problems in children and adolescents: a review of prevalence and concomitant psychological factors. *Int J Paediatr Dent* 17:391–406.

Kurth F, Zilles K, Fox PT, Laird AR, Eickhoff SB (2010). A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. *Brain Struct Funct* 214:519–534.

Litt MD. A model of pain and anxiety associated with acute stressors: distress in dental procedures. *Behav Res Ther* 34:459–476.

Marani E, Usunoff KG (2000). The trigeminal motonucleus in man. *Arch. Physiol. Biochem.* 106:346–354.

Marqueste T, Hug F, Decherchi P, Jammes Y (2003). Changes in neuromuscular function after training by functional electrical stimulation. *Muscle Nerve* 28:181–188.

McGrath PA, Gracely RH, Dubner R, Heft MW (1983). Non-pain and pain sensations evoked by tooth pulp stimulation. *Pain* 15:377–388.

Närhi M, Jyväsjärvi E, Virtanen A, Huopaniemi T, Ngassapa D, Hirvonen T (1992). Role of intradental A- and C-type nerve fibres in dental pain mechanisms. *Proceedings of the Finnish Dental Society. Suomen Hammaslääkäriseuran toimituksia* 88 Suppl 1:507–516.

Netter FH, Dalley AF (2000). Atlas der Anatomie des Menschen. 2., [überarb. und] erw. Ausg. Stuttgart: G. Thieme.

Neugebauer V, Li W (2002). Processing of nociceptive mechanical and thermal information in central amygdala neurons with knee-joint input. *Journal of neurophysiology* 87:103–112.

Neugebauer V, Li W, Bird GC, Han JS (2004). The amygdala and persistent pain. *Neuroscientist* 10:221–234.

Nieuwenhuys R, Voogd J, van Huijzen C (2007). The human central nervous system. 4th ed. Berlin: Springer.

Orchardson R, Gillam DG (2006). Managing dentin hypersensitivity. *Journal of the American Dental Association (1939)* 137:990-8; quiz 1028-9.

Peckham PH, Knutson JS (2005). Functional electrical stimulation for neuromuscular applications. *Annual review of biomedical engineering* 7:327–360.

Peyron R, Laurent B, Garcia-Larrea L (2000). Functional imaging of brain responses to pain. A review and meta-analysis (2000). *Neurophysiologie clinique = Clinical neurophysiology* 30:263–288.

Popovic MR, Keller T (2004). Modular transcutaneous functional electrical stimulation system. *Medical engineering & physics* 27:81–92.

Popovic MR, Popovic DB, Keller T (2002). Neuroprostheses for grasping. *Neurol. Res.* 24:443–452.

Porro CA, Baraldi P, Pagnoni G, Serafini M, Facchin P, Maieron M et al. (2002). Does anticipation of pain affect cortical nociceptive systems? *J. Neurosci.* 22:3206–3214.

Porro CA, Cettolo V, Francescato MP, Baraldi P (2003). Functional activity mapping of the mesial hemispheric wall during anticipation of pain. *NeuroImage* 19:1738–1747.

Ring H, Weingarden H (2007). Neuromodulation by functional electrical stimulation (FES) of limb paralysis after stroke. *Acta Neurochir. Suppl.* 97:375–380.

Rolls ET, O'Doherty J, Kringelbach ML, Francis S, Bowtell R, McGlone F (2003). Representations of pleasant and painful touch in the human orbitofrontal and cingulate cortices. *Cereb. Cortex* 13:308–317.

Scholz J, Woolf CJ (2002). Can we conquer pain? *Nat. Neurosci.* 5 Suppl:1062–1067.

Sessle BJ (1987). The neurobiology of facial and dental pain: present knowledge, future directions. *J. Dent. Res.* 66:962–981.

Sessle BJ (1988). Neurophysiology of orofacial pain. *Dent. Clin. North Am.* 31:595–613.

Sessle BJ (2000). Acute and chronic craniofacial pain. brainstem mechanisms of nociceptive transmission and neuroplasticity, and their clinical correlates. *Crit Rev Oral Biol Med* 11:57–91.

Sessle BJ (2006). Mechanisms of oral somatosensory and motor functions and their clinical correlates. *Journal of oral rehabilitation* 33:243–261.

Sessle BJ (2002). Recent insights into brainstem mechanisms underlying craniofacial pain. *Journal of dental education* 66:108–112.

Sheffler LR, Chae J (2007). Neuromuscular electrical stimulation in neurorehabilitation. *Muscle Nerve* 35:562–590.

Shigenaga Y, Chen IC, Suemune S, Nishimori T, Nasution ID, Yoshida A et al. (1986a). Oral and facial representation within the medullary and upper cervical dorsal horns in the cat. *J. Comp. Neurol.* 243:388–408.

Shigenaga Y, Okamoto T, Nishimori T, Suemune S, Nasution ID, Chen IC et al. (1986b). Oral and facial representation in the trigeminal principal and rostral spinal nuclei of the cat. *J. Comp. Neurol.* 244:1–18.

Shigenaga Y, Otani K, Suemune S (1990). Morphology of central terminations of low-threshold trigeminal primary afferents from facial skin in the cat--intra-axonal staining with HRP. *Brain Res.* 523:23–50.

Sindrup SH, Jensen TS (1999). Efficacy of pharmacological treatments of neuropathic pain: an update and effect related to mechanism of drug action. *Pain* 83:389–400.

Stephan KE, Friston KJ (2010). Analyzing effective connectivity with fMRI. *Wiley interdisciplinary reviews. Cognitive science* 1:446–459.

Sykes LM (2007). Dentine hypersensitivity: a review of its aetiology, pathogenesis and management. *SADJ : journal of the South African Dental Association = tydskrif van die Suid-Afrikaanse Tandheelkundige Vereniging* 62:66–71.

Tracey I, Mantyh PW (2007). The cerebral signature for pain perception and its modulation. *Neuron* 55:377–391.

Usunoff KG, Marani E, Schoen JH (1997). The trigeminal system in man. *Advances in anatomy, embryology, and cell biology* 136:I-X, 1-126.

Virtanen AS (1986). Electrical stimulation of pulp nerves--comparison of monopolar and bipolar electrode coupling. *Pain* 23:279–288.

Virtanen AS, Huopaniemi T, Närhi MV, Pertovaara A, Wallgren K (1987). The effect of temporal parameters on subjective sensations evoked by electrical tooth stimulation. *Pain* 30:361–371.

Vogt BA (2005). Pain and emotion interactions in subregions of the cingulate gyrus. *Nature reviews* 6:533–544.

Weiner DK, Perera S, Rudy TE, Glick RM, Shenoy S, Delitto A (2008). Efficacy of percutaneous electrical nerve stimulation and therapeutic exercise for older adults with chronic low back pain: a randomized controlled trial. *Pain* 140:344–357.

10. ACKNOWLEDGMENTS

First I would like to thank Kai Lutz, Dominik Ettlin, Ashley Barlow, Lutz Jäncke , Sandro Palla and Thierry Keller. They paved the way for starting this project, keeping up the idea of interdisciplinary in a superb manner by combining dental medicine, engineering and neuroscience to create something really new...

Lutz Jäncke for offering me a place in his lab and keeping up a motivational and enthusiastic spirit for all aspects of neuroscience...

All my science colleagues contributing to the superb spirit and cooperation in- and outside the lab...

My family and friends...

And finally: all the volunteers of the past three years, without them, nothing presented in this thesis would have been possible...

11. APPENDIX

Cooperating Institutes:

- GlaxoSmithKline, Consumer Healthcare, Weybridge, UK
- Center for Dental Medicine, Clinic for Removable Prosthodontics, Masticatory Disorders and Special Care Dentistry, University of Zurich
- Automatic Control Laboratory, Swiss Federal Institute of Technology, Zurich
- Institute of Biomedical Engineering, Swiss Federal Institute of Technology and the University of Zurich
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2006 - 2009	**University of Zurich** PhD-student, Department of Psychology, Division Neuropsychology
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